R. K. Razdan, J. Med. Chem., 18, 213 (1975).
(5) The isomeric 1,2-DMH-substituted triol was reported: B. Loev, B. Dienel, M. M. Goodman, and H. Van Hoeven, J. Med. Chem., 17, 1234 (1974).
(6) R. A. Smiley and C. Arnold, J. Org. Chem., 25, 257 (1960).
(7) L. Friedman and H. Shechter, J. Org. Chem., 25, 877 (1960).
(8) F. L. Benton and T. E. Dillon, J. Am. Chem. Soc., 64, 1128 (1942).
(9) E. Vowinkel, Chem. Ber., 95, 2997 (1962).
(10) E. Vowinkel, Angew. Chem., Int. Ed. Engl., 2, 218 (1963).
(11) R. Mechoulam, Ed., "Marijuana", Academic Press, New York, N.Y., 1973, p 3.
(12) R. Adams, C. M. Smith, and S. Loewe, J. Am. Chem. Soc., 63, 1973 (1941).
(13) R. Adams, S. MacKenzie, Jr., and S. Loewe, J. Am. Chem. Soc., 70, 664 (1948).

Semisynthetic Cephalosporins. Synthesis and Structure–Activity Relationships of Analogues with 7-Acyl Groups Derived from 2-(Cyanomethylthio)acetic Acid or 2-[(2,2,2-Trifluoroethyl)thio]acetic Acid and Their Sulfoxides and Sulfones

R. M. DeMarinis,* J. C. Boehm, G. L. Dunn, J. R. E. Hoover, J. V. Uri, J. R. Guarini, L. Phillips, P. Actor, and J. A. Weisbach

Research and Development Division, Smith Kline & French Laboratories, Philadelphia, Pennsylvania 19101.
Received March 15, 1976

The synthesis and in vitro and in vivo activities of a series of cephalosporins having side chains derived from 2-[(2,2,2-trifluoroethyl)thio]acetic acid or 2-(cyanomethylthio)acetic acid and with acetoxymethyl or 3-heterocyclic thiomethyl substituents at the 3 position are described. In both series, increasing the oxidation state of the side-chain sulfur atom from sulfide to sulfoxide/sulfone decreased the in vitro gram-positive activity, but the effect on gram-negative activity was variable and less pronounced. The protective effectiveness in mice infected with *Escherichia coli* increased as the oxidation level of the side-chain sulfur was raised from sulfide to sulfoxide/sulfone. Replacement of the 3-acetoxymethyl by a 3-heterocyclic thiomethyl group resulted in overall improvement of activity both in vitro and in vivo for all oxidation states.

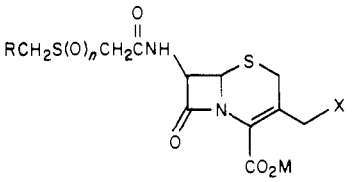
Most of the cephalosporins that possess significant antibacterial activity have on the 7 position an acetamido group to which is attached a heterocyclic or aromatic ring such as phenyl, thiophene, pyridine, or tetrazole. Cephacetrile, which has a simple cyanoacetamido group at the 7 position, is an exception to this.[1] In searching for other potentially useful cephalosporin antibiotics, we have investigated analogues with relatively simple 7-acyl side chains derived from mercaptoacetic acid. Previously, we reported the broad-spectrum activities of 7-trifluoromethylthioacetamido-3-(1-methyl-1*H*-tetrazol-5-ylthiomethyl)-3-cephem-4-carboxylic acid (SK&F 59962, cefazaflur) and closely related analogues.[2] We have also presented the synthesis and structure–activity relationships of 7-sulfonylacetamido-3-cephem-4-carboxylic acids.[3] This article extends the work contained in these earlier papers to analogues where the 7 side chains are derived from 2-(cyanomethylthio)acetic acid and 2-[(2,2,2-trifluoroethyl)thio]acetic acid. Both of these side chains are relatively simple and contain some of the structural features present in either cefazaflur or cephacetrile. The structure–activity relationships described herein compare the effects on activity of varying the oxidation states of the 7 side chain sulfur atom (sulfide, sulfoxide, sulfone) and of varying the 3-substituent (acetoxymethyl, methylthiadiazolethiomethyl, methyltetrazolethiomethyl).

Chemistry. The sulfonylcephalosporins [(**7**, **10**, **13**, **20**, **23**, **26** (Table I)] were synthesized by direct coupling of the side-chain acid to the appropriate *tert*-butyl 7-amino-3-cephem-4-carboxylate.[4] The *tert*-butyl group was subsequently removed with trifluoroacetic acid. The rest of the cephalosporins were prepared by acylating 7-aminocephalosporanic acid (7-ACA) or a 7-amino-3-heterocyclic thiomethyl-3-cephem-4-carboxylic acid. The latter were made by displacement of acetate from 7-ACA with an appropriate thiol by the widely used general procedure[5] described in the Experimental Section.

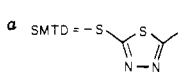
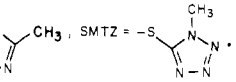
2-(Cyanomethylthio)acetic acid (**1**) was prepared by reacting mercaptoacetic acid with chloroacetonitrile (Scheme I) and characterized as its crystalline potassium salt. The free acid was coupled to *N*-hydroxysuccinimide with DCC in THF to give the activated ester **2**. When this was treated with 1 equiv of *m*-chloroperbenzoic acid, the corresponding sulfoxide **3** was obtained. Both **2** and **3** reacted readily with the triethylamine salts of the 7-amino-3-cephem-4-carboxylic acids in DMF to give cephalosporins **5**, **6**, **8**, **9**, **11**, and **12**. Attempts to oxidize **2** to the sulfone using 2 equiv of *m*-chloroperbenzoic acid were unsuccessful. Consequently, 2-(cyanomethylsulfonyl)acetic acid (**4**) was generated directly from **1** using 2 equiv of *m*-chloroperbenzoic acid. However, attempts to acylate 7-amino-3-cephem-4-carboxylic acid or the corresponding 3-heterocyclic thiomethyl derivatives with this acid via the acid chloride, mixed anhydride, or activated ester were unsuccessful and resulted only in the recovery of starting materials. For the synthesis of **7**, **10**, and **13**, this side-chain acid was coupled to the appropriate *tert*-butyl 7-amino-3-cephem-4-carboxylate using DCC. The *tert*-butyl group was removed with TFA in the presence of a scavenger such as *m*-dimethoxybenzene or acetonitrile in order to trap the *tert*-butyl cation and minimize rearrangement to other positions on the cephalosporin.

2-[(2,2,2-Trifluoroethyl)thio]acetic acid (**14**) was readily obtained by reacting 2,2,2-trifluoroethyl iodide with mercaptoacetic acid[8] (Scheme II). Treatment with thionyl chloride gave the acid chloride. Acylation of the various 7-amino-3-cephem-4-carboxylic acids in aqueous media

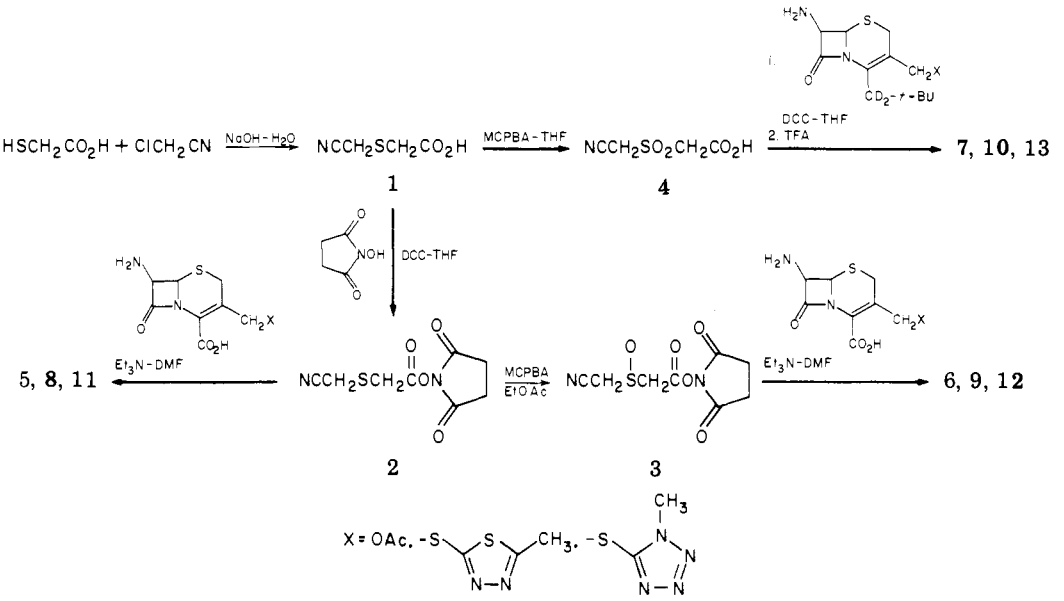
Table I. Structures of Cephalosporin Analogues



Compd	R	*n*	X[a]	M	Formula[b]
5	CN	0	OAc	Na	$C_{14}H_{14}N_3O_6S_2Na$
6	CN	1	OAc	Na	$C_{14}H_{14}N_3O_7S_2Na\cdot1.5H_2O$
7	CN	2	OAc	H	$C_{14}H_{15}N_3O_8S_2\cdot0.5H_2O$
8	CN	0	SMTD	Na	$C_{15}H_{14}N_5O_4S_4Na$
9	CN	1	SMTD	Na	$C_{15}H_{14}N_5O_5S_4Na\cdot H_2O$
10	CN	2	SMTD	H	$C_{15}H_{15}N_5O_6S_4\cdot0.5H_2O\cdot0.25EtOAc$
11	CN	0	SMTZ	Na	$C_{14}H_{14}N_7O_4S_3Na\cdot0.75H_2O$
12	CN	1	SMTZ	Na	$C_{14}H_{14}N_7O_5S_3Na\cdot2H_2O\cdot0.1Et_2O$
13	CN	2	SMTZ	Na	$C_{14}H_{14}N_7O_6S_3Na\cdot1.5H_2O$
18	CF_3	0	OAc	Na	$C_{14}H_{14}F_3N_2O_6S_2Na$
19	CF_3	1	OAc	Na	$C_{14}H_{14}F_3N_2O_7S_2Na\cdot1.75H_2O$[c]
20	CF_3	2	OAc	Na	$C_{14}H_{14}F_3N_2O_8S_2Na$
21	CF_3	0	SMTD	Na	$C_{15}H_{14}F_3N_4O_4S_4Na$
22	CF_3	1	SMTD	Na	$C_{15}H_{14}F_3N_4O_5S_4Na$
23	CF_3	2	SMTD	Na	$C_{15}H_{14}F_3N_4O_6S_4Na$
24	CF_3	0	SMTZ	Na	$C_{14}H_{14}F_3N_6O_4S_3Na\cdot H_2O$
25	CF_3	1	SMTZ	Na	$C_{14}H_{14}F_3N_6O_5S_3Na\cdot0.5H_2O$[d]
26	CF_3	2	SMTZ	Na	$C_{14}H_{14}F_3N_6O_6S_3Na\cdot1.75H_2O$

[a] SMTD =  SMTZ =  [b] Unless otherwise indicated, analytical results for C, H, and N for all compounds were within ±0.4% of the theoretical value. [c] H: calcd, 3.54; found, 3.09. [d] N: calcd, 15.81; found, 15.14.

Scheme I



gave cephalosporins **18**, **21**, and **24**. Oxidation of **14** with 1 equiv of *m*-chloroperbenzoic acid in THF yielded the sulfoxide **15**. This was coupled to *N*-hydroxysuccinimide with DCC to give the crystalline activated ester **16** which was allowed to react with the triethylamine salts of the 7-amino-3-cephem-4-carboxylic acids in dry DMF to give cephalosporins **19**, **22**, and **25**. Activated ester **16** resisted further oxidation to the sulfone. This behavior was similar to that observed for sulfoxide **3** which also bears a strongly electron-withdrawing substituent. The failure of compounds having strongly electron-withdrawing substituents to yield isolable sulfone activated esters is probably due to the extreme lability of these products which does not permit their isolation. In this case acid **14** was first converted to its methyl ester, oxidized with *m*-chloroperbenzoic acid, and hydrolyzed under acidic conditions to give 2-[(2,2,2-trifluoroethyl)sulfonyl]acetic acid (**17**). This was coupled to the *tert*-butyl 7-amino-3-cephem-4-carboxylates using DCC and the intermediate esters were cleaved with TFA–*m*-dimethoxybenzene to give the cephalosporins **20**, **23**, and **26**.

All cephalosporins were characterized and assayed as free acids or as their sodium salts (Table I).

Biology. The in vitro antibacterial activities of the cephalosporins derived from 2-(cyanomethylthio)acetic acid are shown in Table II and those of the analogues derived from 2-[(2,2,2-trifluoroethyl)thio]acetic acid in Table III. The data for these organisms are representative

Scheme II

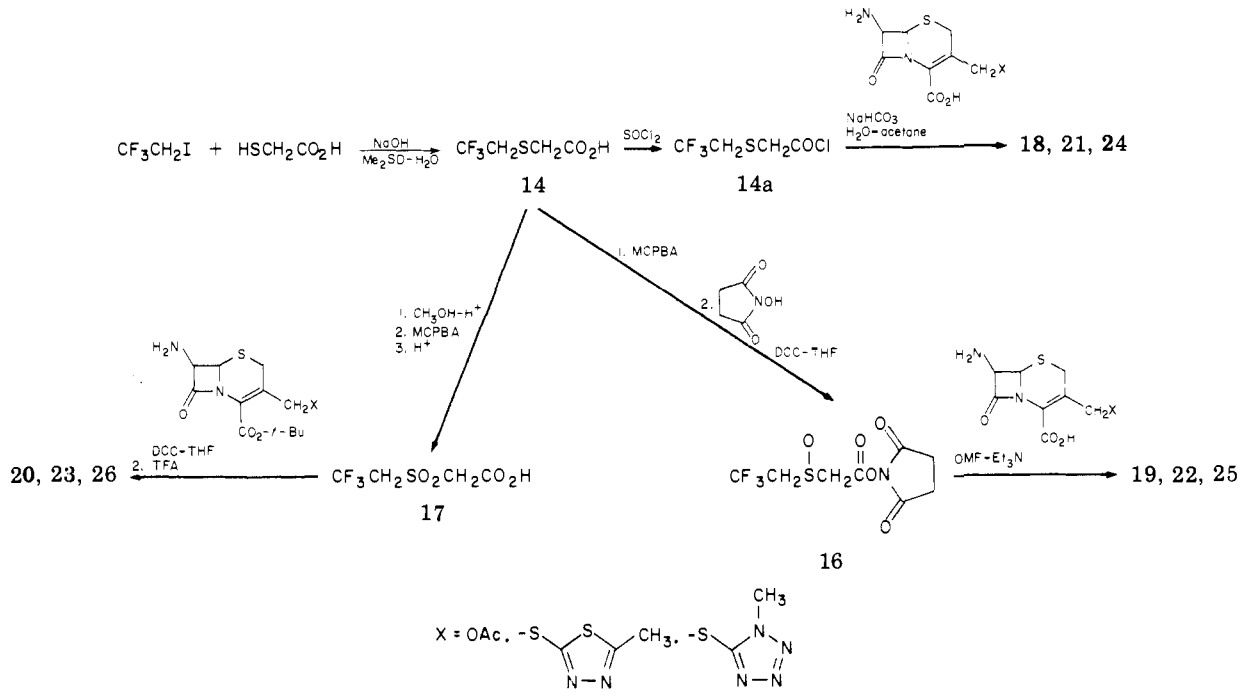
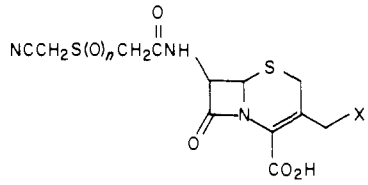


Table II. In Vitro and in Vivo Activities of 7-[2-(Cyanomethylthio)acetamido]-3-cephem-4-carboxylic Acid Analogues

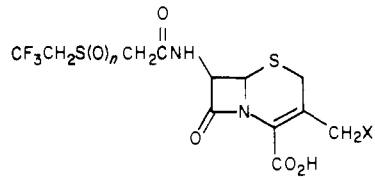


Compound	n	X[d]	Minimum inhibitory concentration, μg/ml[a,b] S.a.(R)[e]	E.c.	K.p.	Sal.p.	Sh.p.	E.a.	Mouse PD_{50} vs. *E. coli* (mg/kg sc)[c]
5	0	OAc	0.2	6.3	1.6	1.6	6.3	>200	>200
6	1	OAc	1.6	12.5	6.3	3.1	6.3	25	21
7	2	OAc	1.6	6.3	25	3.1	6.3	50	35
8	0	SMTD	0.2	1.6	1.6	1.6	1.6	>200	14
9	1	SMTD	0.8	3.1	1.6	0.8	0.8	6.3	22
10	2	SMTD	1.6	6.3	6.3	6.3	3.1	12.5	15
11	0	SMTZ	0.4	0.8	1.6	0.4	0.8	12.5	11
12	1	SMTZ	1.6	1.6	1.6	0.8	1.6	12.5	10
13	2	SMTZ	1.6	1.6	1.6	1.6	1.6	6.3	3
Cefazolin			0.4	0.8	0.8	0.8	0.4	1.6	6
Cephalothin			0.2	3.1	3.1	1.6	6.3	50	50
Cephacetrile			0.4	6.3	3.1	3.1	3.1	6.3	25

[a] The in vitro antibacterial activities are reported as minimum inhibitory concentrations (MIC) in μg/ml. The MIC's were determined by the twofold agar dilution method [T. Jen, B. Dienel, J. Frazee, and J. Weisbach, *J. Med. Chem.*, 15, 1172 (1972)] on Trypticase soy agar buffered to pH 6.0. Organisms selected for inclusion in this table are *S.a.*(R), *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. [b] All compounds reported in this table showed low activity against *Streptococcus faecalis* with MIC values from 13 to >200 μg/ml; therefore, these data are not included. None of the compounds demonstrated significant activity against *Pseudomonas aeruginosa*. [c] The PD_{50} values are expressed as the total dose of compound in mg/kg which afforded protection to 50% of the mice challenged intraperitoneally with approximately 2×10^3 organisms per mouse of an *E. coli* 12140 culture diluted in 5% hog gastric mucin to produce uniformly lethal infections. Fourfold dilutions of each compound were injected subcutaneously in equally divided portions at 1 and 5 h postinfection to five groups of ten mice each. Survivors were observed for 3 days and the mean protective dose (PD_{50}) was calculated by the method of Litchfield and Wilcoxon, *J. Pharmacol. Exp. Ther.*, 96, 99-113 (1949). [d] See footnote *a* to Table I. [e] MIC's were also determined vs. *Staph. aureus* 23390 (Smith) and in all cases the MIC values were within one twofold dilution of those reported for *Staph. aureus* HH 127.

of those obtained from a larger group of four gram-positive and eleven gram-negative bacteria used in our initial screen for evaluating β-lactam antibiotics. Against these organisms both the cyanomethyl and the trifluoroethyl analogues display broad-spectrum antibacterial activity regardless of the oxidation state of the side-chain sulfur atom. Although much of the data is very similar, closer inspection reveals several trends. Displacement of the acetate by various heterocyclic thiols does not produce significant changes in the gram-positive activity in either the cyanomethyl or trifluoroethyl series. This is illustrated by the minimum inhibitory concentrations (MIC's) against a penicillin G resistant strain of *Staphylococcus aureus* [*S.a.*(R)]. In both series these values remain within one

Table III. In Vitro and in Vivo Activities of 7-[2-[(2,2,2-Trifluoroethyl)thio]acetamido]-3-cephem-4-carboxylic Acid Analogues



Compound	n	X[d]	Minimum inhibitory concentration, μg/ml[a,b] S.a.(R)[e]	E.c.	K.p.	Sal.p.	Sh.p.	E.a.	Mouse PD_{50} vs. *E. coli* (mg/kg sc)[c]
18	0	OAc	0.4	6.3	3.1	6.3	6.3	50	184
19	1	OAc	0.8	6.3	3.1	1.6	3.1	6.3	25
20	2	OAc	3.1	25	6.3	3.1	12.5	50	100
21	0	SMTD	0.2	6.3	6.3	6.3	1.6	25	33
22	1	SMTD	0.8	3.1	3.1	1.6	0.8	6.3	6
23	2	SMTD	1.6	6.3	6.3	6.3	6.3	12.5	35
24	0	SMTZ	0.2	1.6	3.1	0.8	0.4	6.3	11
25	1	SMTZ	1.6	0.8	0.4	1.6	0.4	6.3	1.5
26	2	SMTZ	3.1	6.3	3.1	6.3	6.3	50	9
Cefazolin			0.4	0.8	0.8	0.8	0.4	1.6	6
Cephalothin			0.2	3.1	3.1	1.6	6.3	50	50
Cefazaflur			0.4	0.4	0.8	0.4	0.2	1.6	2

[a-e] See corresponding footnotes to Table II.

twofold dilution of the parent 3-acetoxymethyl analogues for each oxidation state of the side-chain sulfur atom. However, against gram-negative bacteria, this structural change results in general improvement of the antibacterial activity. In this analogue sequence (acetoxymethyl, methylthiadiazolethiomethyl, methyltetrazolethiomethyl), the methyltetrazolethiomethyl analogue is the most active. This agrees with previous observations in our laboratories that the methyltetrazolethiomethyl derivative is frequently the most active of a series of analogues.[2]

Increasing the oxidation state of the side-chain sulfur atom from sulfide to sulfoxide/sulfone decreases the gram-positive activity in stepwise fashion. The changes observed in the levels of gram-negative activity are more variable and less pronounced. In general, the sulfones are less active than the corresponding sulfide and sulfoxide analogues. Exceptions (especially in the cyanomethyl series) are seen with the activities against *Enterobacter*. It is interesting that in the trifluoroethyl series the gram-negative activities of the sulfoxides are frequently better than those of the corresponding sulfide and sulfone analogues.

The mouse infection–protection activities (PD_{50} values) against *Escherichia coli* are also shown in Tables II and III. For each oxidation state, those derivatives having a 3-acetoxymethyl group generally display the poorest PD_{50} values. For these cephalosporins, replacement of acetoxymethyl by heterocyclic thiomethyl improves in vivo effectiveness, with the analogues having a methyltetrazolethiomethyl substituent being the most active. Variation of the oxidation state of the side-chain sulfur produces an interesting effect on the protective efficacy of these compounds. While the in vitro activity decreases or remains the same upon going from sulfide to sulfone, the in vivo effectiveness increases in most cases. The sulfoxides also are more active in vivo than the corresponding sulfides, especially in the trifluoroethyl examples, even when this is not reflected in the in vitro activities. These effects might be related to differences in pharmacokinetic handling of these compounds in the mouse.

Of the cephalosporins described here, the 3-methyltetrazolethiomethyl analogues having at the 7 position 2-(cyanomethylthio)acetic acid (compound **11**) or 2-[2,2,2-trifluoroethyl)sulfinyl]acetic acid (compound **25**) display the best combination of in vitro and in vivo activities. Although these cephalosporins display good broad-spectrum antimicrobial activities in vitro, and they afford protection in vivo at relatively low dosage levels, cefazaflur continues to display the best combination of activities in this group.

Experimental Section

Melting points were determined in open capillary tubes using a Thomas-Hoover Uni-Melt apparatus. Unless indicated otherwise, ir spectra were obtained in Nujol mull using a Perkin-Elmer Infracord; NMR spectra were obtained in Me_2SO-d_6 or Me_2SO-d_6–D_2O on a Varian T-60 spectrometer using Me_4Si as an internal standard. The ir and NMR data of all compounds were consistent with structure. Where elemental analyses are indicated only by symbols of the elements, analytical results obtained for these elements were within ±0.4% of the theoretical values. $MgSO_4$ was used as drying agent for organic extracts. The yields of final purified cephalosporins ranged from 3 to 54%. The heterocyclic thiols used in this work were prepared by procedures described in the literature.[6,7]

7-Amino-3-heterocyclic Thiomethyl-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 0.23 mol of $NaHCO_3$ in 200 ml of water, and the resultant solution was heated to 40–50 °C. The appropriate thiol (0.13 mol) in 200 ml of acetone was added and the solution was stirred under reflux. Periodically a solid sample was isolated by adjusting the pH of an aliquot of the reaction mixture to 3.5. The reaction was judged to be complete when the ir spectrum showed no acetoxy remaining (~4 h). The pH was maintained between 7.4 and 7.8 by the addition of 5% $NaHCO_3$ or 3 N HCl if necessary. When the reaction was complete the solution was cooled in an ice bath and acidified with 3 N HCl to pH 3.5. The resulting precipitate was collected, washed with water and acetone, and dried. The crude products were purified by suspending in water and adding 6 N HCl until solution was effected. The acidic solution (Norit) was held at room temperature for 30 min to 5 h depending on the presence of residual 7-ACA (NMR) in the crude product. After filtering through Supercel, the filtrate was cooled and adjusted to pH 3.5 with 20% NaOH. The precipitate was washed with water and acetone and dried. These intermediates were used without further purification.

2-(Cyanomethylthio)acetic Acid (1). In 200 ml of H_2O was dissolved 16 g (0.4 mol) of NaOH and 18.4 g (0.2 mol) of mercaptoacetic acid. The solution was cooled to room temperature,

15 g (0.2 mol) of chloroacetonitrile was added in one portion, and the resulting two-phase system stirred until solution was effected (~2 h). The resulting solution was extracted with 200 ml of EtOAc (which was discarded), acidified to pH 1.5 with HCl, and extracted with three 100-ml portions of EtOAc. The combined extracts were washed, dried, and evaporated to give 22 g (85%) of pale yellow oil. The potassium salt was formed for analysis by adding a solution of the acid in 50 ml of anhydrous EtOH to an equimolar solution of KOH in 200 ml of EtOH. The resulting precipitate was collected and dried to give a 77% yield of white crystals: mp 212 °C dec with darkening at >205 °C; NMR (D_2O) δ 3.42 (s, 2), 3.6 ppm (s, 2). Anal. ($C_4H_4NO_2SK$) C, H, N.

***N*-Hydroxysuccinimidyl 2-(Cyanomethylthio)acetate (2).** A solution of 19.6 g (0.15 mol) of acid 1, 17.4 g (0.15 mol) of *N*-hydroxysuccinimide, and 30 g (0.15 mol) of DCC in 150 ml of THF was stirred at room temperature overnight. The precipitated urea was removed by filtration and the filtrate evaporated to give a solid. Crystallization from $CHCl_3$ yielded 24 g (71%) of white crystals: mp 90–91 °C; NMR δ 2.85 (s, 4), 3.90 (s, 2), 4.05 ppm (s, 2). Anal. ($C_8H_8N_2O_4S_2$) C, H, N.

***N*-Hydroxysuccinimidyl 2-(Cyanomethylsulfinyl)acetate (3).** A suspension of 22.8 g (0.1 mol) of **2** in 150 ml of EtOAc was treated dropwise with 20.2 g (0.1 mol) of 85% *m*-chloroperbenzoic acid in 150 ml of EtOAc. About 30 min after the addition was complete, a clear solution formed. The solution was stirred at room temperature overnight and evaporated to leave a white hygroscopic crystalline residue. This was extracted with three 200-ml portions of Et_2O which were discarded. The residue was taken up in 50 ml of hot THF and refrigerated. It deposited crystals which were filtered and dried under vacuum in a desiccator: NMR δ 2.90 (s, 4), 4.55 ppm (m, 4). The product (13 g, 56%) is extremely hygroscopic and hydrates extensively upon even very brief exposure to the atmosphere.

2-(Cyanomethylsulfonyl)acetic Acid (4). A cooled solution of 26.2 g (0.2 mol) of 1 in 400 ml of THF was treated dropwise with 81 g (0.4 mol) of 85% *m*-chloroperbenzoic acid in 400 ml of THF. When addition was complete, the solution was warmed to room temperature and stirred overnight. The solvent was removed to leave a solid residue which was extracted with three 300-ml portions of distilled water. The extracts were combined and refrigerated for 2 days after which they were filtered from precipitate and lyophilized to give 28.3 g (87%) of off-white solid: NMR δ 4.56 (s, 2), 5.05 (s, 2), 11.1 ppm (s, 1). Anal. ($C_4H_5NO_4S$) C, H, N.

7-[2-(Cyanomethylthio)acetamido]- and 7-[2-(Cyanomethylsulfinyl)acetamido]cephalosporins (5, 6, 8, 9, 11, 12). Triethylamine was added dropwise to a stirred suspension of 10 mmol of the appropriate 7-amino-3-cephem-4-carboxylic acid in 50 ml of dry DMF until solution was completed. The activated ester (10 mmol) was added in one portion and the resulting solution was stirred at room temperature for 1.5 h. The DMF was evaporated and the residue partitioned between 150 ml of EtOAc and 150 ml of H_2O. The pH was adjusted to 6.8 and the organic extract separated and discarded. The aqueous phase was layered with fresh EtOAc and adjusted to pH 2.0 with 3 N HCl. An emulsion usually formed which was broken by filtration through a pad of Celite. The filtrate layers were separated and the aqueous phase was extracted twice more with EtOAc. The combined extracts were dried and evaporated to give the cephalosporin. If the cephalosporin was not solid, it was dissolved in CH_3OH and titrated to pH 7.0 with 5% $NaOCH_3$ in CH_3OH. Et_2O was added dropwise with rapid stirring to precipitate the sodium salt of the cephalosporin which was collected and dried under vacuum.

2-[(2,2,2-Trifluoroethyl)sulfinyl]acetic Acid (15). To a cooled solution of 8.7 g (50 mmol) of acid 14 in 50 ml of dry THF was added dropwise 10.1 g (50 mmol) of 85% *m*-chloroperbenzoic acid in 50 ml of THF. When addition was complete, the solution was warmed to room temperature and stirred for 4 h. The THF was evaporated and the residue triturated with three 100-ml portions of water. The aqueous extracts were filtered and evaporated leaving a solid residue which was dissolved in 150 ml of EtOAc, dried, filtered, and reduced to a volume of ~20 ml. This was added dropwise to 400 ml of rapidly stirred petroleum ether and the resulting precipitate collected and dried to give 7.1 g (75%) of white solid: mp 102–104 °C; NMR δ 4.05 ppm (complex multiplet). Anal. ($C_4H_5F_3O_3S$) C, H.

***N*-Hydroxysuccinimidyl 2-[(2,2,2-Trifluoroethyl)sulfinyl]acetate (16).** A solution of 6.65 g (35 mmol) of acid **14**, 4.03 g (35 mmol) of *N*-hydroxysuccinimide, and 7.22 g (35 mmol) of DCC in 150 ml of dry THF was stirred at room temperature for 2.5 h. The precipitated urea was removed and the filtrate evaporated to leave 8.8 g (88%) of off-white solid. Recrystallization from benzene gave white crystals: mp 124 °C (darkening at 116 °C); NMR (acetone-d_6) δ 2.9 (s, 4), 4.2 ppm (m, 4). Anal. ($C_8H_8NO_5F_3S \cdot 0.25H_2O$) C, H, N.

2-[(2,2,2-Trifluoroethyl)sulfonyl]acetic Acid (17). A solution of 52.1 g of acid **14** in 250 ml of dry MeOH containing a catalytic amount of *p*-toluenesulfonic acid was refluxed overnight. The MeOH was evaporated and the oil partitioned between H_2O and Et_2O. The combined extracts were washed with 5% $NaHCO_3$ and saturated NaCl, dried, and evaporated to give 45.3 g (80%) of colorless liquid: NMR (CCl_4) δ 3.25 (q, 2, J = 10 Hz), 3.35 (s, 2), 3.75 ppm (s, 3). To 45.3 g (0.24 mol) of the methyl ester in 150 ml of $CHCl_3$ was added dropwise 97.4 g (0.48 mol) of 85% *m*-chloroperbenzoic acid in 500 ml of Et_2O. When addition was complete, the reaction was warmed to room temperature and stirred overnight. The solution was extracted with saturated $NaHSO_3$, 5% $NaHCO_3$ until basic, and saturated NaCl, dried, and evaporated to give 26 g (49%) of crystalline sulfone: NMR δ 3.90 (s, 3), 4.55 (s, 2), 4.80 ppm (q, 2, J = 10 Hz). This was dissolved in a mixture of 50 ml of MeOH and 60 ml 3 N HCl and was refluxed overnight. It was cooled, diluted with 200 ml of water, and extracted with five 200-ml portions of EtOAc, and the combined extracts were back-extracted with 5% $NaHCO_3$. The basic, aqueous phase was acidified to pH 1.5 and extracted with EtOAc. The combined acidic extracts were dried and evaporated to give 17.3 g (78%) of crystalline acid: mp 145 °C [sublimed 140 °C (0.25 mm)]; NMR δ 4.5 (s, 2), 4.7 (q, 2, J = 10 Hz), 6.5 ppm (broad, 1). Anal. ($C_4H_5F_3O_4S$) C, H.

7-[[2-(2,2,2-Trifluoroethyl)thio]acetamido]cephalosporins (18, 21, 24). In 100 ml of water containing 25 mmol of $NaHCO_3$ was dissolved 10 mmol of the appropriate 7-amino-3-cephem-4-carboxylic acid. An equal volume of acetone was added and the solution was cooled to −20 °C. To this rapidly stirred solution was added dropwise over 15 min 12 mmol of 2-[(2,2,2-trifluoroethyl)thio]acetyl chloride in 50 ml of acetone. When addition was complete, the solution was stirred in the cold for 30 min and then at room temperature for 1 h. Throughout the reaction, the pH was maintained at ~7.5 by the addition of $NaHCO_3$ or HCl if necessary. The solution was diluted with 100 ml of H_2O and extracted with three 50-ml portions of Et_2O (discarded). The aqueous phase was layered with 150 ml of EtOAc and adjusted to pH 1.5 with 3 N HCl. The acidified aqueous phase was extracted twice more with EtOAc and the combined extracts were dried and evaporated. the residue was taken up in ~30 ml of CH_3OH and adjusted to pH 7.0 with 5% $NaOCH_3$ in CH_3OH. Et_2O was added dropwise to precipitate the product which was collected and dried under vacuum.

7-[2-(Cyanomethylsulfonyl)acetamido]cephalosporins (7, 10, 13). A solution of 10 mmol of the *tert*-butyl 7-amino-3-cephem-4-carboxylate, 10 mmol of 2-(cyanomethylsulfonyl)acetic acid, and 10 mmol of DCC in 150 ml of dry THF was stirred at room temperature overnight. The precipitated urea was removed by filtration and the filtrate evaporated to a gum. This was dissolved in EtOAc and washed with 1 N HCl, 5% NaOH, and H_2O. The compounds were chromatographed over silica gel, eluting with benzene–EtOAc (1:1 compound **7**) or EtOAc (compounds **10**, **13**). The purified *tert*-butyl ester was dissolved in 10 ml of CH_3CN and treated with 10 ml of TFA. After standing for 4 h at room temperature compound **7** crystallized from the solution and was isolated by filtration. Cephalosporins **10** and **13** were isolated by adding the TFA solution dropwise to a rapidly stirred flask of Et_2O. The resulting precipitates were collected, washed with Et_2O, and dried. The precipitates were taken up in EtOAc, washed well with H_2O, dried, and evaporated to give the products. Conversion of **13** to its salt was effected by the addition of sodium 2-ethylhexanoate (30% in 2-propanol) to a methanolic solution of the free acid. Dropwise addition of Et_2O gave the product which was collected and dried.

2-[(2,2,2-Trifluoroethyl)thio]acetyl Chloride (14a). The acid **14** was prepared in 98% yield by reaction of mercaptoacetic

acid with 2,2,2-trifluoroethyl iodide.[5] The acid chloride was formed by stirring the acid overnight in excess thionyl chloride. Excess reagent was removed under vacuum and the residue distilled to give 90–100% of acid chloride **14a** as a pale yellow liquid, bp 46–48 °C (10 mm).

7-[[2-(2,2,2-Trifluoroethyl)sulfinyl]acetamido]cephalosporins (19, 22, 25). Triethylamine was added dropwise to a stirred suspension of 10 mmol of the appropriate 7-amino-3-cephem-4-carboxylic acid in 50 ml of dry DMF until solution was complete. The activated ester (10 mmol) was added in one portion and the resulting solution was stirred at room temperature for 1.5 h. The DMF was evaporated and the residue partitioned between 150 ml of EtOAc and 150 ml of H_2O. The pH was adjusted to 6.8 and the organic extract separated and discarded. The aqueous phase was layered with fresh EtOAc and adjusted to pH 2.0 with 3 N HCl. An emulsion usually formed which was broken by filtration through a pad of Celite. The filtrate layers were separated and the aqueous phase was extracted twice more with EtOAc. The combined extracts were dried and evaporated to give the cephalosporin. If the cephalosporin was not solid, it was dissolved in CH_3OH and titrated to pH 7.0 with 5% $NaOCH_3$ in CH_3OH. Et_2O was added dropwise with rapid stirring to precipitate the sodium salt of the cephalosporin which was collected and dried under vacuum.

7-[[2-(2,2,2-Trifluoroethyl)sulfonyl]acetamido]cephalosporins (20, 23, 26). A solution of 10 mmol of the *tert*-butyl 7-amino-3-cephem-4-carboxylate, 10 mmol of 2-[(2,2,2-trifluoroethyl)sulfonyl]acetic acid, and 10 mmol of DCC in 150 ml of dry THF was stirred at room temperature overnight. The precipitated urea was removed by filtration and the filtrate evaporated to a gum. This was dissolved in a solution of 20 ml of TFA and 20 ml of *m*-dimethoxybenzene and stirred at room temperature for 2 h. It was added dropwise to 300 ml of rapidly stirred ether and the resulting precipitate collected, washed with ether, and dried. The cephalosporin was converted to its sodium salt by dissolving it in 20 ml of MeOH and adjusting the pH to 7.0 with 5% $NaOCH_3$ in CH_3OH. The product was precipitated by the dropwise addition of Et_2O.

Note **Added in Proof.** Several other laboratories have recently reported studies on some of the chemical and biological properties of compound 11.[9,10]

Acknowledgment. We wish to thank Mr. John Zarembo and his staff in the Analytical & Physical Chemistry Department of Smith Kline & French Laboratories for the elemental analyses. We are also grateful to Mr. William M. Bryan for the synthesis of compounds **18** and **23**.

References and Notes

(1) F. Knüsel, E. A. Konopka, J. Gelzer, and A. Rosselet, *Antimicrob. Agents Chemother., 1970*, 140 (1971).
(2) R. M. DeMarinis, J. R. E. Hoover, G. L. Dunn, P. Actor, J. V. Uri, and J. A. Weisbach, *J. Antibiot.*, **28**, 6, 463 (1975).
(3) R. M. DeMarinis, J. R. E. Hoover, L. L. Lam, J. V. Uri, J. R. Guarini, L. Phillips, P. Actor, and J. A. Weisbach, *J. Med. Chem.*, **19**, 754 (1976).
(4) 7-ACA *tert*-butyl ester was prepared according to R. J. Stedman, *J. Med. Chem.*, **9**, 444 (1966). The 3-heterocyclic thiomethyl-3-cephem-4-carboxylic acid esters were prepared by treating a suspension of the corresponding acid in CH_2Cl_2 with a threefold molar excess of O-*tert*-butyl diisopropylpseudourea: B. M. Bain and E. P. Tiley, Belgian Patent 755 505 (1970).
(5) C. F. Murphy and J. A. Webber, "Cephalosporins and Penicillins, Chemistry and Biology", E. H. Flynn, Ed., Academic Press, New York, N.Y., 1972, Chapter 4.
(6) J. Goerdeler, J. Ohm, and O. Tegtmeyer, *Chem. Ber.*, **89**, 1534 (1956).
(7) F. Dersch and R. H. Clark, U.S. Patent 2 465 149 (1949).
(8) R. Pinson, Jr., E. C. Schreiber, E. H. Wiseman, J. Chiaini, and D. Baumgartner, *J. Med. Pharm. Chem.*, **5**, 491 (1962).
(9) H. Breuer, M. D. Treuner, D. Isaacson, and H. H. Gadebusch, Abstracts of the 9th International Congress of Chemotherapy, London, July 13–18, 1975.
(10) G. J. Miraglia, H. Basch, P. Lukaszow, K. J. Renz, and H. H. Gadebusch, *J. Antibiot.*, **29**, 8, 860 (1976).

11,12-Secoprostaglandins. 1. Acylhydroxyalkanoic Acids and Related Compounds

John B. Bicking,* Charles M. Robb, Robert L. Smith, Edward J. Cragoe, Jr.,

Merck Sharp & Dohme Research Laboratories, West Point, Pennsylvania 19486

Frederick A. Kuehl, Jr., and Lewis R. Mandel

Merck Institute for Therapeutic Research, Rahway, New Jersey 07065. *Received April 15, 1976*

The synthesis is described of a series of acylhydroxyalkanoic acids which embody structural modifications of that class of secoprostaglandins which are formally derived from the natural substances by scission of the cyclopentane ring between carbon atoms 11 and 12. These analogues have been tested for their ability to stimulate cAMP formation in the mouse ovary, a characteristic action of the (*E*)-prostaglandins, and for their ability to bind to the rat lipocyte prostaglandin receptor. Certain members of the series that most closely resemble the prostaglandins in structure (e.g., 8-acetyl-12-hydroxyheptadecanoic acid) markedly stimulate cAMP formation at concentrations in the pharmacological range and show a significant affinity for the prostaglandin receptor. Conversely, these compounds are not substrates for prostaglandin 15-hydroxydehydrogenase which catalyzes a major reaction in the biological deactivation of the prostaglandins.

A practical objective of prostaglandin analogue research is the development of a group of compounds with adequate metabolic stability and differing tissue specificities so that the numerous biological actions of the prostaglandins[1] can, in effect, be separated and applied in the treatment of various diseases. Our work in this field has centered on compounds that may be termed 11,12-secoprostaglandins since they are formally derived from the prostaglandins by cleavage of the C-11 to C-12 bond of the cyclopentane ring. Ring opening of prostaglandin E_1 in this manner gives, for example, a branched chain alkanoic acid 8-(*R*)-(3-hydroxypropionyl)-12(*S*)-hydroxy-10-*trans*-heptadecenoic acid (**1**).

During studies on the synthesis of 11,12-secoprostaglandins such as **1**, it was discovered that the simpler analogue, 8-acetyl-12-hydroxyheptadecanoic acid (**2a**), possesses a number of the biological actions of the natural prostaglandins. The synthesis and biological examination