OXIDATION STUDIES ON β-LACTAM ANTIBIOTICS: IN-VITRO ANTIMICROBIAL ACTIVITY OF THE OXIDIZED PRODUCTS OF 3-HETEROARYLTHIOMETHYL-CEPH-3-EMS

Rajeshwar SINGH*, Maya P. SINGH and Ronald G. MICETICH

SynPhar Laboratories Inc., No. 24 Taiho Alberta Center, No. 24, 4290-91A St., Edmonton, Alberta, Canada T6E 5 V2

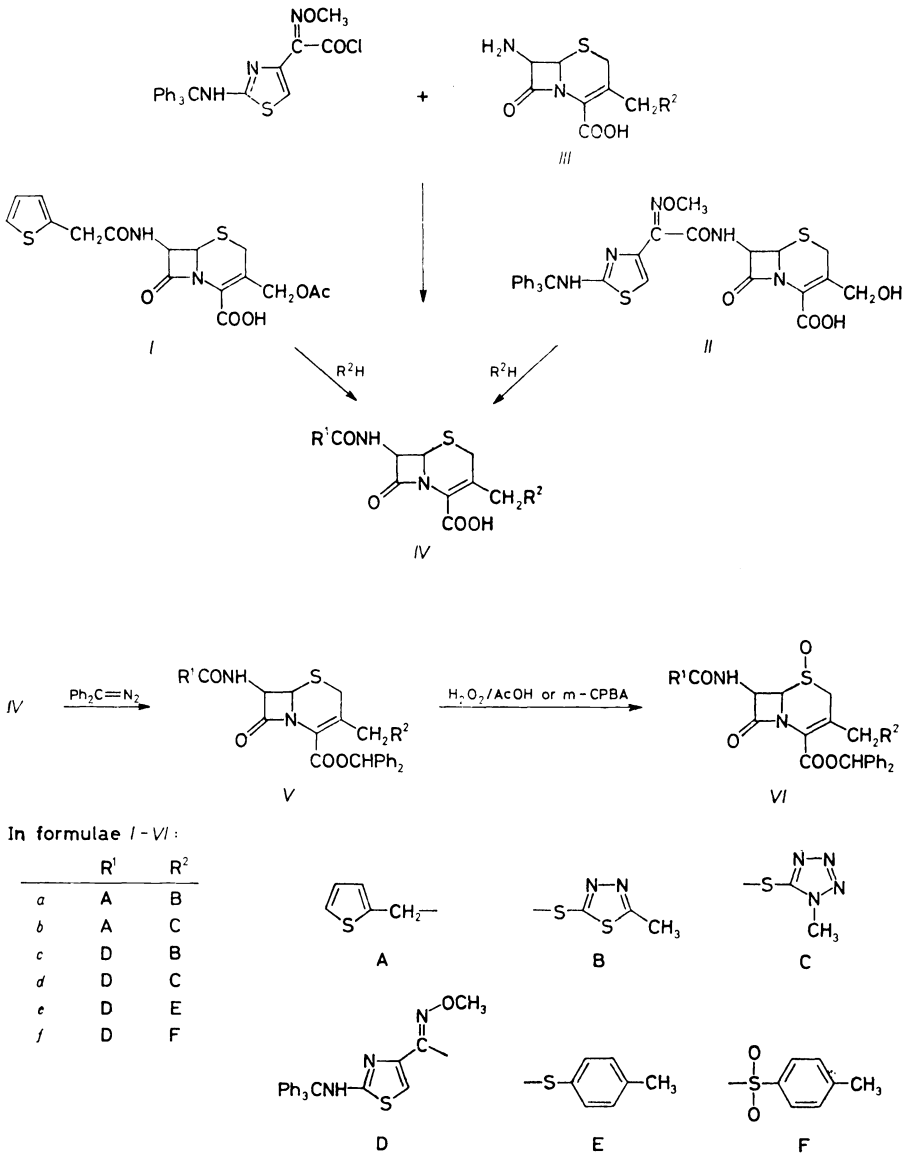
Received October 15, 1990
Accepted January 3, 1991

Dedicated to Dr Miroslav Protiva on the occasion of his 70th birthday.

Various products from the oxidation of 3-heteroarylthiomethyl-ceph-3-ems using *m*-chloroperbenzoic acid (*m*-CPBA) and hydrogen peroxide in acetic acid in varying stoichiometric ratios have been isolated, identified and their in vitro antimicrobial activity determined. The oxidized compounds with the 2-aminothiazol-4-yl-(*Z*)-2-methoxyiminoacetamido side chain showed better antibacterial activity against various Gram negative bacteria compared to the unoxidized compounds.

In recent years the sulfoxides and sulfones of certain cephalosporins have been reported to possess antibiotic activity[1,2]. In continuation of our studies[3], we have investigated the oxidation pattern of 3-heteroaryl thiomethylcephems[4-7]. These compounds contain two thioether functions, each of which is susceptible to oxidation to a sulfoxide (*R* and *S* isomers) and sulfone. We obtained a number of products from oxidations using both *m*-CPBA and hydrogen peroxide in acetic acid as oxidants, in varying stoichiometry. The chemistry and the antimicrobial activity of the various oxidation products is reported in this paper.

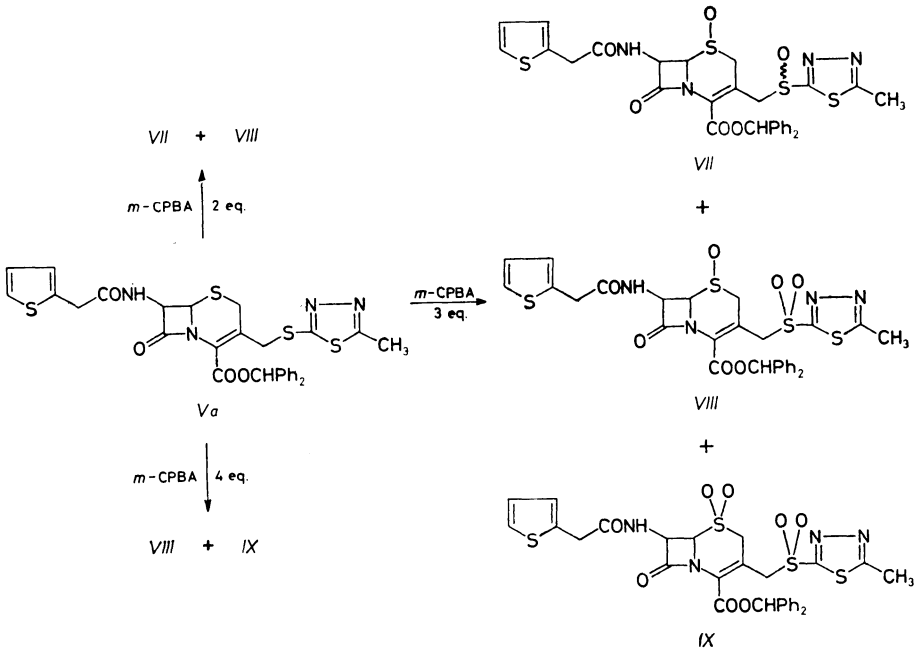
Our initial target was the synthesis of various oxidized products of the 7β-[2-(2-aminothiazol-4-yl)-(*Z*)-2-methoxyiminoacetamido]-3-heteroarylthiomethyl-ceph-3--em-4-carboxylic acid; the starting compounds are broad spectrum cephalosporins[2]. Oxidations using varying stoichiometry of oxidizing agents (hydrogen peroxide in acetic acid or *m*-CPBA) to cephem compound were carried out using diphenylmethyl 7β-thiophen-2-acetamido-3-[2-methyl-1,3,4-thiadiazol-5-yl)-thiomethyl]-ceph--3-em-4-carboxylate *Va* which was prepared[8] by the nucleophilic substitution of the acetoxy group of the 3-acetoxymethylcephem-4-carboxylic acid with 5-mercapto-2--methyl-1,3,4-thiadiazole in phosphate buffer followed by esterification with diphenyldiazomethane (Scheme 1).



SCHEME 1

Oxidation of *Va* with one equivalent of *m*-CPBA or hydrogen peroxide in acetic acid gave the monosulfoxide *VIa* whereas with two equivalents of *m*-chloroperbenzoic acid, the disulfoxide *VII* (mixture of *R* and *S* isomer at C_3—CH_2S) and the sulfoxide-sulfone *VIII* were produced; with three equivalents of *m*-CPBA, a mixture

of compounds *VII*, *VIII* and *IX* was obtained; and oxidation with four equivalents of *m*-CPBA gave compounds *VIII* and *IX* (Scheme 2). Oxidation of *Vb* with one equivalent of *m*-CPBA or hydrogen peroxide in acetic acid gave compound *VIb* and further oxidation gave decomposed products.

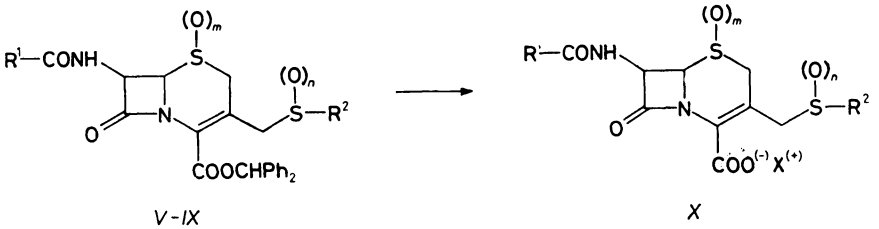


SCHEME 2

The various starting compounds, diphenylmethyl 7β-[2-(2-tritylaminothiazol-4-yl)--(*Z*)-2-methoxyiminoacetamido]-3-heteroarylthiomethyl-ceph-3-em-4-carboxylates *Vc* to *Vf* were prepared by either of the following methods: (i) condensation[9] of 2-(trityl-aminothiazol-4-yl)-(*Z*)-2-methoxyimino acetic acid with 7β-amino-3-heteroarylthio-methyl-ceph-3-em-4-carboxylic acid[10] *III* using PCl_5, $NaHCO_3$, acetone, CH_2Cl_2 and water; (ii) reaction[11] of the heteroaryl mercaptan with 7β-[2-(2-tritylamino-thiazol-4-yl)-2-methoxyiminoacetamido-3-hydroxymethyl-ceph-3-em-4-carboxylic acid *II* (Scheme 1). Thus compounds *IVc*, *IVd* and *IVf* obtained, on esterification with diphenyldiazomethane, gave compounds *Vc*, *Vd* and *Vf*. Compounds *Vc* and *Vd* on oxidation with one equivalent of hydrogen peroxide in acetic acid or *m*-CPBA gave the respective sulfoxides *VIc* and *VId*, each of which on further oxidation with *m*-CPBA gave a complex mixture of decomposed products.

The hydrolysis of the diphenylmethyl ester of the cephems *V*–*IX* were carried out in trifluoroacetic acid with anisole at 0°C, and gave the corresponding acids which

were converted into their sodium or potassium salt *X* by sodium potassium 2-ethyl hexanoate (Scheme 3).



V-IX *X*

In formulae *V-X*: $m = 0,1$; $n = 1,2$; X = Na, K

SCHEME 3

Antibacterial Properties and Structure–Activity Relationships

The in vitro antibacterial activity of compounds *Xa*–*Xm* (Table I) against Gram positive and Gram negative aerobic bacteria were determined by the agar dilution method. Cephalothin and cephotaxime (also synthesized in our laboratory) were used as reference compounds. The compounds *Xc*–*Xm* have two different types of side chain at C-7 position – one similar to that of cephalothin and the other similar to cefotaxime. Antimicrobial spectra (Table I) of the unoxidized and oxidized cephalosporins suggest that the oxidation of the sulfur of the ring and of the C-3 side-chain reduces the antimicrobial activity of compounds having the thiophene acetamido side-chain (*Xa*, *Xk*, *Xl*, *Xm*, *Xb*, *Xh*) significantly against both Gram positive and Gram negative organisms with the exception of compound *Xg* which has the ring sulfur oxidized to sulfoxide stage. This compound shows slightly improved antibacterial activity against a few selected Gram negative organisms such as *E. cloacae*, *P. vulgaris*, *P. rettigeri*, *M. morganii* and *S. marcescens* over the unoxidized compound *Xa* (Table I). Such an effect was not observed with the compounds *Xb* and *Xh* which have a different mercaptoheteroaryl substituent (tetrazole instead of thiadiazole) at the C-3 position. On the other hand, in compounds having an aminothiazolylmethoxyiminoacetamido side-chain (*Xc*, *Xd*, *Xi*, *Xj*, *Xe* and *Xf*), oxidation of sulfur provides compounds with potent activity against Gram negative organisms whereas they retain moderate activity against Gram positive organisms. The activity is slightly reduced against Gram positive organisms but is increased up to 5-fold against most of the Gram negative organisms except *Pseudomonas aeruginosa* (compared *Xc* vs *Xi* and *Xd* vs *Xj*). The considerable increase in antibacterial activity of oxidized cephalosporins with the cefotaxime type side chain at the C-7 position is probably due to either their facilitated penetration through the Gram negative cell wall or due to their increased stability towards β-lactamases present

TABLE I

In vitro antimicrobial activity of some cephalosporins and their oxidation products *X*

Organism[b]		MIC[a], μg/ml														
		Xa	*Xb*	*Xc*	*Xd*	*Xe*	*Xf*	*Xg*	*Xh*	*Xi*	*Xj*	*Xk*	*Xl*	*Xm*	CET[c]	CTX[d]
S.pn.	A9505	0·06	0·06	ND	ND	ND	ND	1	2	ND	ND	4	8	8	0·13	0·016
S.p.	A9604	0·06	0·13	0·16	0·016	0·008	0·03	1	2	0·06	0·06	4	8	8	0·03	0·008
S.f.	A20688	32	125	>125	>125	>125	>125	>125	125	>125	>125	125	>125	>125	32	>125
S.a.	A9537	2	0·25	0·25	1	1	4	2	4	2	8	32	32	16	0·13	1
S.a.	A9537[e]	2	2	8	8	>125	32	32	>63	63	>125	>63	>63	>63	1	4
S.a.	A9606[f]	16	>125	1	2	1	4	16	>125	8	8	>125	>125	>125	4	2
S.a.	A15097[g]	>125	>125	125	125	>125	>125	>125	>125	>125	>125	>125	>125	>125	>125	>125
E.c.	A15119	4	4	0·25	0·06	4	2	8	16	0·13	0·06	16	32	125	8	0·06
E.c.	A20341—1	16	8	0·25	0·06	8	2	16	63	0·06	0·06	32	8	>125	16	0·13
K.pn.	A9664	8	16	1	1	32	8	16	32	0·50	0·50	63	16	16	9	0·25
K.pn.	A20468	125	125	2	1	32	8	125	>125	1	1	>125	>125	>125	125	0·50
E.cl.	A9659	>125	>125	0·25	0·5	8	8	125	>125	0·06	0·06	>125	>125	>125	>125	2
E.cl.	A9656	>125	>125	32	32	125	63	63	>125	0·50	1	>125	>125	>125	>125	32
P.m.	A9900	1	1	0·03	0·016	2	0·25	8	16	0·03	0·06	16	4	32	1	0·03
P.v.	A21559	>125	63	63	63	>125	63	63	>125	0·13	0·25	>125	>125	>125	125	0·50
M.m.	A15153	>125	125	0·06	0·06	4	1	125	>125	0·06	0·06	>125	63	>125	>125	0·06
P.r.	A22424	>125	>125	1	0·25	4	4	125	>125	0·13	0·13	>125	>125	>125	>125	0·13
S.m.	A20019	>125	>125	2	2	16	16	63	>125	0·25	0·25	>125	>125	>125	125	4
P.a.	A9843	>125	>125	125	63	>125	125	>125	>125	>125	>125	>125	>125	>125	>125	16
P.a.	A21213	>125	>125	63	63	>125	>125	125	>125	>125	>125	>125	>125	>125	>125	32

[a] Determined by serial dilution in Mueller–Hinton agar, inoculum 10^6 cfu/ml, incubation at 35°C for 18 h; [b] S.pn. *Streptococcus pneumoniae*, S.p. *Streptococcus pyogenes*, S.f. *Streptococcus faecalis*, S.a. *Staphylococcus aureus*, E.c. *Escherichia coli*, K.pn. *Klebsiella pneumoniae*, E.cl. *Enterobacter cloacae*, P.m. *Proteus mirabilis*, P.v. *Proteus vulgaris*, M.m. *Morganella morganii*, P.r. *Providencia rettgeri*, S.m. *Serratia marcescens*, P.a. *Pseudomonas aeruginosa*, ND not determined; [c] CET Cephalothin; [d] CTX Cefotaxime; [e] 50% serum; [f] Penicillin resistant; [g] Methicillin resistant.

in the periplasma. Further work has been undertaken to investigate these speculations. From the antibacterial activity of several compounds synthesized and tested in our laboratory, it is clear that the substitutions at C-3 and C-7 positions along with the oxidation stages of the sulfur of the ring and the C-3 side-chain have noticeable effects on the antimicrobial activity of the cephem derivatives.

EXPERIMENTAL

Melting points were taken on a Unimelt Thomas Hoover capillary melting point apparatus and are uncorrected. Infrared spectra were obtained using Nicolet DX-FTIR (in KBr, cm^{-1}). The ^1H NMR spectra (δ, ppm; J, Hz) were recorded on a Bruker AM-300 instrument with TMS as internal standard. Minimum inhibitory concentration (MIC) against Gram positive and Gram negative microorganisms were determined by the agar dilution method.

7β-(Thiophen-2-acetamido)-3-[(2-methyl-1,3,4-thiadiazol-5-yl)thiomethyl]-ceph-3-em-4-carboxylic Acid (*IVa*)

A solution of 7β-(thiophen-2-acetamido)-3-acetoxymethyl-ceph-3-em-4-carboxylic acid (1·18 g; 0·03 mol), $NaHCO_3$ (0·509 g; 0·06 mol) and 5-mercapto-2-methyl-1,3,4-thiadiazole (0·438 g; 0·035 mol) in phosphate buffer (25 ml) of pH 6·4 was stirred for 5 h at 60°C. The reaction mixture was cooled and acidified to pH 3·0 with 3M-HCl and extracted with ethyl acetate. The organic extract was washed with water and brine, dried over Na_2SO_4 and concentrated. The residue was redissolved in a minimum volume of ethyl acetate and precipitated with the addition of hexane, to provide the title compound *IVa*, yield 850 mg (62%).

7β-[2-Tritylaminothiazol-4-yl)-(*Z*)-2-methoxyiminoacetamido]-3-[(1-methyl-1,2,3,4-tetrazol-5-yl)thiomethyl]-ceph-3-em-4-carboxylic Acid (*IVd*)

Method A. Phosphorus pentachloride (2·08 g; 0·01 mol) was added to an ice-cold solution of 2-(2-tritylaminothiazol-4-yl)-(*Z*)-2-methoxyimino acetic acid (4·44 g; 0·01 mol) and triethylamine (1·41 ml; 0·01 mol) in 70 ml of CH_2Cl_2. The mixture was stirred in an ice-cold bath for 20 min, then evaporated to dryness. The residue was dissolved in a mixture of 50 ml of CH_2Cl_2 and 50 ml of acetone and evaporated. Acetone (50 ml) was added to the residue and the mixture was filtered. The filtrate was added to an ice-cold solution of 7β-amino-3-[(1-methyl-1,2,3,4-tetrazol-5-yl)thiomethyl]-ceph-3-em-4-carboxylic acid (2·64 g; 0·008 mol) in 50 ml of acetone and 75 ml of water containing sodium bicarbonate (0·84 g; 0·01 mol) and triethylamine (2·82 ml; 0·02 mol). The mixture was stirred in the cold for 30 min followed by room temperature for 1 h and then acidified with 4M-HCl to pH 2. Water was added and the mixture was extracted with ethyl acetate (3 × 50 ml). The combined extract was washed with water and brine, dried over Na_2SO_4 and concentrated to obtain 5·63 g (93%) of the desired product *IVd*. IR (KBr): 3 222, 3 041, 2 943, 1 791, 1 730, 1 624, 1 597, 1 526, 150, 752, 705. ^1H NMR (CD_3SOCD_3): 3·60 and 3·70 ABq, 1 H (2-CH_2, $J = 18$); 3·82 s, 3 H (NCH_3); 3·93 s, 3 H ($NOCH_3$); 4·23 and 4·38 ABq, 2 H (3-CH_2); 5·10 d, 1 H (H-6, $J = 5$); 5·72 dd, 1 H (H-7, $J = 5$; $J = 8$); 6·72 s, 1 H (CH); 7·34 m, 15 H (aromatic protons); 8·90 s, 1 H (NH); 9·62 d, 1 H (NH, $J = 8$).

Similarly compounds *IVc*, *IVe* and *IVb* have been prepared from the corresponding starting material.

Method B: A solution of methyl O-phenylene phosphate (372 mg) in CH_2Cl_2 was added to a solution of N-methylpyrrolidine 7β-[2-(2-tritylaminothiazol-4-yl)-(*Z*)-2-methoxyiminoaceta-

mido]-3-hydroxymethyl-ceph-3-em-4-carboxylate (*II*, 740 mg; 1 mmol) and 5-mercapto-1--methyl-1,2,3,4-tetrazole (100mg; 1·15 mmol) in 20 ml of CH_2Cl_2 and cooled to −45°C. The reaction mixture was stirred for 2·5 h between −5°C to −15°C and then the solution was concentrated. The residue was diluted with water, acidified with diluted HCl and extracted with 50 ml of ethyl acetate. The extract was washed with water and brine solution, dried over Na_2SO_4 and concentrated to give the required compound *IVd*, yield 700 mg (93%). The spectral data is the same as described above.

Diphenylmethyl 7β-(thiophen-2-acetamido)-3-[(2-methyl-1,3,4-thiadiazol-5--yl)thiomethyl]-ceph-3-em-4-carboxylate (*Va*). General Procedure

A solution of diphenyldiazomethane in CH_2Cl_2 (25 ml) was added dropwise to a solution of compound *IVa* (4·68 g; 0·01 mol) in CH_2Cl_2 (100 ml) and methanol (to dissolve compound *IVa*) until the red colour disappeared, and the reaction mixture was stirred at room temperature for 2 h, then concentrated on a rotary evaporator. The residue was dissolved in ethyl acetate and washed successively with $NaHCO_3$, water and brine. The organic phase was dried over Na_2SO_4, concentrated and the residue chromatographed over silica gel using CH_2Cl_2–EtOAc (50 : 50) as eluant, to obtain the desired compound *Va* in 50% yield. IR: 3 284, 3 029, 1 784, 1 724, 1 688, 1 529.

Ester Vb: yield 57%; IR: 3 296, 3 050, 2 951, 1 785, 1 722, 1 685, 1 526.
Ester Vc: yield 74%; IR: 3 377, 3 041, 2 943, 1 700, 1 727, 1 605, 1 526.
Ester Vd: yield 54%; IR: 3 296, 3 050, 2 935, 1 790, 1 728, 1 603, 1 526.
Ester Ve: yield 60%; IR: 3 296, 3 050, 2 935, 1 707, 1 728, 1 602, 1 524.
Ester Vf: yield 62%; IR: 3 353, 3 050, 2 935, 1 788, 1 730, 1 606, 1 526.

Oxidation of Ester *V* with H_2O_2–CH_3COOH. General Procedure

A mixture of 30% H_2O_2 (1·36 g; 0·012 mol) and acetic acid (2·40 g; 0·04 mol) was added slowly to a stirred solution of compound *V* (0·01 mol) in 35 ml of CH_2Cl_2. The reaction mixture was stirred at room temperature for 24 h, washed successfully with water, $NaHCO_3$ solution and brine, dried over Na_2SO_4 and concentrated. The residue was chromatographed over silica gel using EtOAc–CH_2Cl_2 (50 : 50) as eluant, and compound *VI* was obtained.

Sulfoxide VIa: yield 68%; IR: 3 377, 3 033, 2 935, 1 793, 1 725, 1 685, 1 512. ^{1}H NMR ($CDCl_3$): 2·66 s, 3 H (CH_3); 3·46 and 4·00 ABq, 2 H (2-CH_2, J = 19); 3·86 s, 2 H (CH_2); 4·00 and 4·64 ABq, 2 H (3-CH_2, J = 14); 4·46 d, 1 H (H-6, J = 5); 6·06 dd, 1 H (H-7, J = 5; J = 8); 7·00—7·54 m, 15 H (NH, aromatic and thiophene protons).

Sulfoxide VIb: yield 73%; IR: 3 370, 3 050, 2 951, 1 796, 1 725, 1 687, 1 511.
Sulfoxide VIc: yield 82%; IR: 3 377, 3 041, 2 943, 1 800, 1 728, 1 687, 1 516.
Sulfoxide VId: yield 63%; IR: 3 371, 3 045, 2 937, 1 800, 1 726, 1 688, 1 520.

Oxidation of compound *Va* with one equivalent of *m*-CPBA gave the same compound *VIa* in 60% yield.

Oxidation of Ester *Va* with Two Equivalents of *m*-Chloroperbenzoic Acid

m-Chloroperbenzoic acid (0·884 g; 0·004 mol; 78% active oxygen) was added slowly to an ice-cold solution of *Va* (1·268 g; 0·002 mol) in CH_2Cl_2 within 5 min. The reaction mixture was stirred at 5—10°C for 2·5 h, separated solid was filtered and washed with CH_2Cl_2. The filtrate was washed successively with $NaHCO_3$ solution, water and brine, dried over Na_2SO_4 and concen-

trated. The residue was chromatographed over silica gel using EtOAc–CH_2Cl_2 (1 : 1) as eluant and two fractions were obtained.

Fraction A (VIII): 160 mg (12%); IR: 3 371, 2 930, 1 799, 1 720, 1 688, 1 510. ^{1}H NMR ($CDCl_3$): 2·74 s, 3 H (CH_3); 3·62 and 3·88 ABq, 2 H (2-CH_2, J = 10); 3·84 s, 2 H (CH_2); 4·26 and 5·40 ABq, 2 H (3-CH_2, J = 14); 4·56 d, 1 H (H-6, J = 5); 6·06 dd, 1 H (H-7, J = 5; J = 8); 6·80 s, 1 H (CH); 6·96—7·48 m, 14 H (aromatic and thiophene protons).

Fraction B (VII, mixture of *R* and *S* isomer at C-3); 800 mg (60%); IR: 3 370, 3 050, 2 935, 1 794, 1 725, 1 685, 1 511. ^{1}H NMR ($CDCl_3$): 2·665 s, 1·8 H (CH_3); 2·74 s, 1·2 H (CH_3); 3·14 and 3·70 ABq, 0·8 H (2-CH_2, J = 18); 3·42 and 3·78 ABq, 1·2 H (2-CH_2, J = 18); 3·84 bs, 1·2 H (3-CH_2); 3·96 and 5·18 ABq, 0·8 H (3-CH_2, J = 13·5); 4·50 d, 1 H (H-6, J = 5); 6·04 dd, 0·4 H (H-7, J = 5; J = 8); 6·06 dd, 0·6 H (H-7, J = 5; J = 8); 6·80—7·54 m, 15 H (aromatic and thiophene protons).

Oxidation of Ester *Va* with Four Equivalents of *m*-Chloroperbenzoic Acid

m-Chloroperbenzoic acid, 78% active oxygen (2·66 g; 0·012 mol) was added to a solution of *Va* (1·90 g; 0·003 mol) in CH_2Cl_2. The reaction mixture was stirred for 6 h at room temperature and worked up as described above. The residue was chromatographed over silica gel using EtOAc–CH_2Cl_2 (1 : 1) as eluant, and two fractions were obtained.

Fraction A (IX): 340 mg (17%); IR: 3 386, 2 943, 1 808, 1 731, 1 691, 1 514, 1 339, 1 134, 702. ^{1}H NMR ($CDCl_3$): 2·70 s, 3 H (CH_3); 3·86 s, 2 H (CH_2); 3·96 and 4·36 ABq, 2 H (2-CH_2, J = 18); 4·38 and 5·16 ABq, 2 H (3-CH_2, J = 13·5); 5·04 d, 1 H (H-6, J = 5); 6·20 dd, 1 H

TABLE II

Physical constants of cephalosporins *X*

Compound[a]	R^1	R^2	X	*m*	*n*	Yield, %	m.p., °C[b]
Xa	A	B	Na	0	0	70	162
Xb	A	C	Na	0	0	63	125
Xc	D	B	K	0	0	67	197
Xd	D	C	K	0	0	68	184
Xe	D	E	K	0	0	63	—
Xf	D	E	K	0	2	68	—
Xg	A	B	Na	1	0	65	173
Xh	A	C	Na	1	0	66	175
Xi	D	B	K	1	0	80	215
Xj	D	C	K	1	0	68	200
Xk	A	B	Na	1	1[c]	54	150—167
Xl	A	B	Na	1	2	67	196—197
Xm	A	B	Na	2	2	69	150

[a] For explanation of substituents A, B, C, D see Scheme 1; [b] decomposition; [c] mixture of *R* and *S* isomers.

TABLE III

Spectral properties of cephalosporins *X*

Compound	IR	^{1}H NMR (D_2O)
Xa	3 280, 2 960, 1 763, 1 671, 1 604	2·42 (3 H, s, CH_3); 3·04 and 3·40 (2 H, ABq, J = 18, 2-CH_2); 3·56 and 3·64 (2 H, ABq, J = 16, CH_2); 3·70 and 4·16 (2 H, ABq, J = 14, 3-CH_2); 4·74 (1 H, d, J = 5, H-6); 5·34 (1 H, d, J = 5, H-7); 6·74 (2 H, m, thiophene protons); 7·04 (1 H, m, thiophene proton)
Xb	3 422, 2 962, 1 765, 1 687, 1 609	3·76 and 4·06 (2 H, ABq, J = 18, CH_2); 4·20 and 4·28 (2 H, ABq, J = 16, 2-CH_2); 4·35 (3 H, s, N—CH_3); 4·42 and 4·65 (2 H, ABq, J = 14, 3-CH_2); 5·40 (1 H, d, J = 5, H-6); 5·97 (1 H, d, J = 5, H-7); 7·38 (2 H, m, thiophene); 7·68 (1 H, m, thiophene)
Xc	3 320, 3 189, 2 943, 1 767, 1 680, 1 606, 1 532	2·72 (3 H, s, CH_3); 3·64 and 3·84 (2 H, ABq, J = 18, 2-CH_2); 3·95 and 4·50 (2 H, ABq, J = 14, 3-CH_2); 4·00 (3 H, s, OCH_3); 5·17 (1 H, d, J = 5, H-6); 5·79 (1 H, d, J = 5, H-7); 7·02 (1 H, s, thiazole)
Xd	3 314, 3 203, 2 937 1 768, 1 675, 1 606, 1 533	3·50 and 3·84 (2 H, ABq, J = 18, 2-CH_2); 4·02 (3 H, s, N—CH_3); 4·06 (3 H, s, OCH_3); 4·08 and 4·35 (2 H, ABq, J = 14, 3-CH_2); 5·20 (1 H, d, J = 5, H-6); 5·80 (1 H, d, J = 5, H-7); 7·02 (1 H, s, thiazole)
Xe	3 353, 3 172, 2 935, 1 765, 1 680, 1 606, 1 533	2·08 (3 H, s, CH_3); 3·06 and 3·44 (2 H, ABq, J = 17·5, 2-CH_2); 3·44 and 4·16 (2 H, ABq, J = 14, 3-CH_2); 3·80 (3 H, s, OCH_3); 4·80 (1 H, d, J = 4·5, H-6); 5·52 (1 H, d, J = 4·5, H-7); 6·78 (1 H, s, thiazole); 6·96 (2 H, d, J = 12 *m*-phenyl;) 7·12 (2 H, d, J = 12, *o*-phenyl)
Xf	3 328, 3 197, 2 960, 1 767, 1 677, 1 615, 1 535	2·30 (3 H, s, CH_3); 3·20 and 3·60 (2 H, ABq, J = 18, 2-CH_2); 3·84 (3 H, s, OCH_3); 4·00 and 5·08 (2 H, ABq, J = 14, 3-CH_2); 5·04 (1 H, d, J = 4·5, H-6); 5·62 (1 H, d, J = 4·5, H-7); 6·84 (1 H, s, thiazole), 7·32 (2 H, d, J = 12, *m*-phenyl); 7·60 (2 H, d, J = 12, *o*-phenyl)
Xg	3 240, 2 952, 1 772, 1 671, 1 612	2·72 (3 H, s, CH_3); 3·52 and 3·68 (2 H, ABq, J = 18, 2-CH_2) 3·62 and 4·48 (2 H, ABq, J = 14, 3-CH_2); 3·70 and 3·78 (2 H, ABq, J = 16, CH_2); 4·64 (1 H, d, J = 5, H-6); 5·62 (1 H, d, J = 5, H-7); 6·04 (2 H, m, thiophene; 7·20 (1 H, m, thiophene)
Xh	3 364, 2 982, 1 778, 1 675, 1 615, 1 519	3·84—4·06 (8 H, m, NCH_3, CH_2, 2-CH_2, 3-CH); 4·54 (1 H, d, J = 14, 3-CH); 4·88 (1 H, d, J = 5, H-6); 5·88 (1 H, d, J = 5, H-7); 7·06 (2 H, m, thiophene); 7·40 (1 H, m, thiophene)

TABLE III
(*Continued*)

Compound	IR	^{1}H NMR (D_2O)
Xi	3 328, 3 197, 2 967, 1 761, 1 659, 1 616, 1 523	2·74 (3 H, s, CH_3); 3·82 and 3·97 (2 H, ABq, J = 18, 2-CH_2); 3·84 and 4·72 (2 H, ABq, J = 14, 3-CH_2); 4·02 (3 H, s, OCH_3); 4·96 (1 H, d, J = 5, H-6); 5·97 (1 H, d, J = 5, H-7); 7·02 (1 H, s, thiazole)
Xj	3 320, 3 205, 2 943, 1 780, 1 674, 1 612, 1 533	3·82 and 3·96 (2 H, ABq, J = 18, 2-CH_2); 4·01 and 4·54 (2 H, ABq, J = 14, 3-CH_2); 4·02 (3 H, s, N—CH_3); 4·04 (3 H, s, OCH_3); 5·00 (1 H, d, J = 5, H-6); 6·00 (1 H, d, J = 5, H-7); 7·02 (1 H, s, thiazole)
Xk (mixture of *R* and *S* isomer)	3 394, 2 951, 1 779, 1 678, 1 614, 1 516	2·68 (3 H, s, CH_3); 3·34 (0·4 H, d, J = 18, 2-CH); 3·60—3·84 (3·6 H, m, 2-CH_2, CH_2); 4·10 (0·4 H, d, J = 14, 3-CH); 4·20 (0·6 H, d, J = 14, 3-CH); 4·70 (1·6 H, m, 3-CH, H-6); 4·90 (0·4 H, d, J = 14, 3-CH); 5·64 (1 H, d, J = 5, H-7); 6·86 (2 H, m, thiophene); 7·20 (1 H, m, thiophene)
Xl	3 380, 2 963, 1 783, 1 672, 1 626, 1 524	2·66 (3 H, s, CH_3); 3·62 and 3·78 (2 H, ABq, J = 18, 2-CH_2); 3·66 and 3·74 (2 H, ABq, J = 16, CH_2); 4·22 and 5·30 (2 H, ABq, J = 14, 3-CH_2); 4·74 (1 H, d, J = 5, H-6); 5·60 (1 H, d, J = 5, H-7); 6·88 (2 H, m, thiophene); 7·16 (1 H, m, thiophene)
Xm	3 386, 3 287, 2 951, 1 790, 1 684, 1 634, 1 532	2·66 (3 H, s, CH_3); 3·66 and 3·72 (2 H, ABq, J = 18, CH_2); 3·90 and 4·32 (2 H, ABq, J = 18, 2-CH_2); 4·34 and 5·05 (2 H, ABq, J = 14, 3-CH_2); 5·18 (1 H, d, J = 5, H-6); 5·66 (1 H, d, J = 5, H-7); 6·80 (2 H, m, thiophene, 7·14 (1 H, m, thiophene)

(H-7, J = 5; J = 8); 6·78 s, 1 H (CH); 6·98 m, 2 H (thiophene-H); 7·34 m, 12 H (aromatic and thiophene protons).

Fraction B (VIII): 430 mg (21%); IR: 3 370, 2 927, 1 798, 1 720, 1 626, 1 508. ^{1}H NMR is the same as described at oxidation with 2 equivalents of *m*-chloroperbenzoic acid.

Sodium 7β-(Thiophen-2-acetamido)-3[(2-methyl-1,3,4-thiadiazol-5-yl)thiomethyl]-ceph-3-em--4-carboxylate (*Xa*). General Procedure

To a solution of diphenylmethyl 7β-(thiophen-2-acetamido)-3-[(2-methyl-1,3,4-thiadiazol-5-yl)-thiomethyl]-ceph-3-em-4-carboxylate (1 g) in anisole (2 ml) was added trifluoroacetic acid (8 ml) with ice cooling. The reaction mixture was stirred at 0—5°C for 20 min and poured onto a cold mixture (40 ml) of diethyl ether and hexane (1 : 2). The precipitate formed was filtered, dissolved in ethyl acetate (if required dissolved in minimum amount of dry methanol) and pH was adjusted to 7·0 with a methanolic solution of sodium 2-ethyl hexanoate. The solid separated out after addi-

tion of ether was filtered, washed with ether (5 × 50 ml) and dried, thus the desired compound *Xa* was obtained.

Compounds *Vb*—*Vf* and *VIa*—*VId* were deesterified by a similar method. In the case of *Vc*—*Vf* and *VIc*—*VId*, the time required was two hours to complete the reaction. The yields, m.p. and spectral data of compounds *X* are given in Table II and Table III.

The authors appreciate the help of Dr R. B. Morin of Bristol Myers, U.S.A., in assisting with the antimicrobial testing.

REFERENCES

1. Koning J. J., Marx A. F., Poot M. M., Smid P. M., Verweij J. in: *Recent Advances in the Chemistry of β-Lactam Antibiotics* (K. Elks, Ed.), p. 161. The Royal Society of Chemistry, London 1976.
2. Durckheimer W., Klesel N., Limbert M., Schinner E., Seeger K., Seliger H. in: *Recent Advances in the Chemistry of β-Lactam Antibiotics* (G. I. Gregory, Ed.), p. 46. The Royal Society of Chemistry, London 1980.
3. Micetich R. G., Singh R., Maiti S. N.: Heterocycles *22*, 531 (1984).
4. Singh M. P., Singh R., Spevak P., Maiti S. N., Micetich R. G. in: *Abstracts of Papers of 28th Intersci. Conf. on Antimicrob. Agents Chemother., Los Angeles 1988*; p. 183.
5. Ref.[4], p. 184.
6. Singh R., Singh M. P., Micetich R. G.: Indian J. Chem., B, in press.
7. Lee M., Micetich R. G., Singh R., Spevak P., Singh M. P., Maiti S. N.: Magn. Reson. Chem. *26*, 719 (1988).
8. Takamo T., Kurita M., Nikaido H., Meora M., Konishi N., Nakagawa R. (Fugisawa Pharmaceutical Co., Osaka, Japan): U.S. 3516996, June 23, 1970.
9. Curran W. V., Ross A. A.: J. Antibiot. *36*, 179 (1983).
10. Lewis G. S., Nelson P. H.: J. Med. Chem. *22*, 1214 (1979).
11. Ochial M., Morimoto A., Miyawaki T., Matsushita Y., Okada T., Natsugari H., Kida M.: J. Antibiot. *34*, 171 (1981).