Structures of the Cephalosporolides B—F, A Group of C_{10} Lactones from *Cephalosporium aphidicola*

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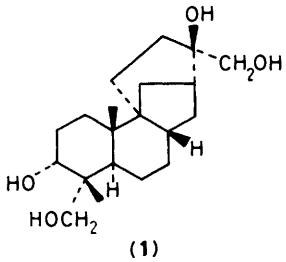
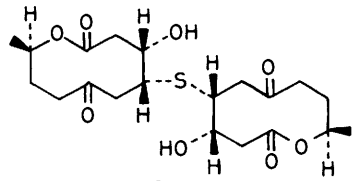
The structures of the pentaketides lactones, the cephalosporolides B—F, were elucidated by a combination of spectroscopic, chemical, and *X*-ray analyses.

The fungus, *Cephalosporium aphidicola*, produces the diterpenoid, aphidicolin (**1**).[1] In the course of our biosynthetic studies on aphidicolin[2] we have elucidated the structures of a number of other metabolites of the fungus including a bisdecanolide, thiobiscephalosporolide A (**2**).[3] In this paper we report the structures of a group of lactones, the cephalosporolides B—F, (**3**), (**4**) and (**7**)—(**9**) which were produced by an industrial fermentation of the fungus *C. aphidicola*, ACC 3490. These compounds are related to the recently described diplodialides [*e.g.* (**10**)] produced by *Diplodia pinea*,[4] and the pyrenolides [*e.g.* (**11**)] produced by *Pyrenophora teres*.[5]

Cephalosporolide B, $C_{10}H_{14}O_4$, M^+ 198 (**3**) showed i.r. absorptions at 3 320 cm^{-1} (OH), 1 730 and 1 260 cm^{-1} (COO), 1 690 and 1 640 cm^{-1} (αβ-unsaturated ketone) and u.v. absorption at 215 nm (ε 5 080). The ^{13}C n.m.r. spectrum (see Table 1) contained ten resonances which, on the basis of their chemical shift and multiplicity in the SFORD spectrum, were assigned to one methyl group, three methylenes, two CH–O groups, one –CH=CH–, one COO and one C=O grouping. The ^1H n.m.r. spectrum (see Table 2) confirmed the presence of the methyl group [δ 1.25 (d, *J* 6.5 Hz)], a secondary lactone (δ 5.05), a low-field secondary alcohol (δ 5.25) and an αβ-unsaturated ketone (δ 5.80 and 6.23). ^1H N.m.r. spin decoupling studies (for coupling constants see Table 3), established several part structures. Irradiation of the methyl group doublet (δ 1.25) converted the multiplet resonances at δ 5.05 into a double doublet (*J* 4 and 7 Hz). Irradiation at δ 5.05 collapsed the methyl doublet to a singlet and modified a two-proton multiplet (δ 2.05) suggesting the presence of the part structure (**12**). The key to a second part structure came from the irradiation of a double doublet at δ 2.95 (*J* 6 and 15 Hz). This led to the collapse of a multiplet at δ 5.22 to a broadened triplet (*J* 9 Hz) and to perturbation of a multiplet at δ 2.45. Irradiation at δ 5.22 not only collapsed the double doublet at δ 2.95 to a doublet (*J* 15 Hz) and modified the three-proton multiplet at δ 2.45, but also reduced the double doublet at δ 5.8 (*J* 9 and 12 Hz) to a doublet (*J* 12 Hz). Irradiation at δ 5.8 confirmed the coupling to the resonance at δ 5.22 and to the second olefinic proton resonance at δ 6.23 (*J* 12 Hz). These ^1H decoupling experiments were best accommodated by part structure (**13**) and suggested the overall structure, 3-hydroxy-6-oxodec-4-en-9-olide (**3**) for the metabolite. This structure was confirmed by inter-relationship with thiobiscephalosporolide-A (*vide infra*). Spectroscopic information concerning the configuration of the double bond was ambiguous. The magnitude of the olefinic coupling constant lies between the commonly accepted[6] ranges for *cis*- and *trans*-olefins. Consequently an *X*-ray analysis (see Figure 1) was carried out on the highly crystalline methanesulphonate (**5**). This revealed a *cis* configuration for the double bond. Application of Horeau's method[7] to the determination of the absolute stereochemistry of the hydroxy group showed that it possessed the '*S*' configuration. Thus structure (**3**) represents the overall stereochemistry of the lactone.


(**1**)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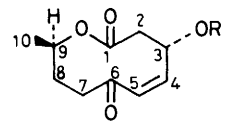
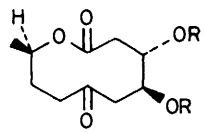

(**3**) R = H
(**5**) R = Ms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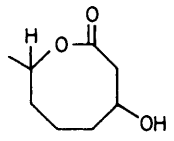
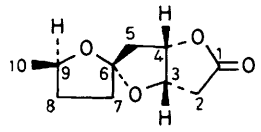
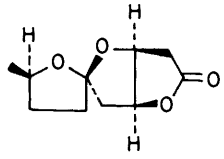
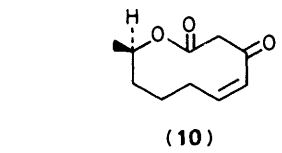
(**6**) R = Ms


(**7**)


(**8**)


(**9**)

Cephalosporolide C, $C_{10}H_{16}O_5$, M^+ 216 (**4**) showed a broad hydroxy absorption at 3 400 cm^{-1} and a lactone absorption at 1 725 and 1 250 cm^{-1}. However, it lacked the i.r. and u.v. absorption of the αβ-unsaturated ketone of cephalosporolide B. The ^{13}C n.m.r. spectrum (Table 1) contained signals that were attributed to a methyl group, four methylene groups, three –CH·O– groups, and a lactone and a carbonyl group. ^1H N.m.r. spin decoupling studies established several part structures from which the overall structure was deduced. Irradiation of the methyl group doublet (δ 1.27) and the multiplet resonance at δ 5.13 resulted in changes in the spectrum consistent with the part structure (**12**). Irradiation at the multiplet at δ 4.25 removed a



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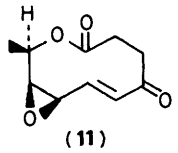

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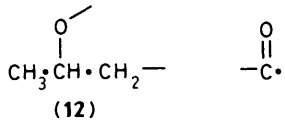

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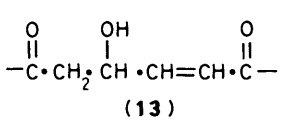
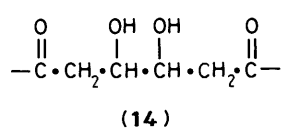


(13)



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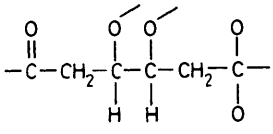

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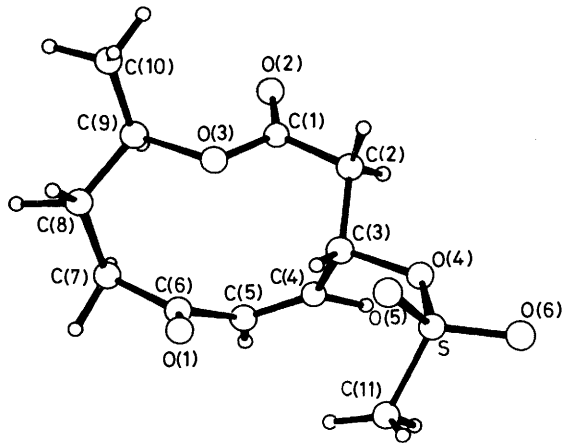


Figure 1. Structure of cephalosporolide B 3-*O*-methanesulphonate (**5**)

small coupling (3 Hz) from a double doublet at δ 2.93 (*J* 3 and 18 Hz) and a large coupling (*J* 12 Hz) from a further double doublet (*J* 12 and 18 Hz) at 2.35 and modified a multiplet at δ 3.44. Irradiation of the latter signal collapsed a two-proton signal at δ 2.75 to a doublet and affected the multiplet at δ 4.25. Further irradiation at δ 2.93 resulted in the collapse of the signal at δ 2.35 and 4.25 revealing a coupling of 10 Hz between the signals at δ 4.25 and 3.44. This led to the part structure (**14**). The magnitude (10 Hz) of the coupling between the protons of the vicinal glycol indicated a *trans* relationship in this system. The overall structure, 3,4-dihydroxy-6-oxodecan-9-olide (**4**) which followed from these partial structures was confirmed by *X*-ray analysis (see Figure 2) and by an inter-relationship with thiobiscephalosporolide A.

The cephalosporolides B and C were inter-related with thiobiscephalosporolide A (**2**) in the following way. Oxidation of compound (**2**) with sodium metaperiodate in acetic acid gave the corresponding sulphoxide. Unlike thiobiscephalosporolide A, this formed only a monomethanesulphonate on treatment with methanesulphonyl chloride in pyridine, possibly because of the involvement of one hydroxy group in hydrogen bonding with the sulphoxide. Pyrolysis of the sulphoxide led to the isolation of the 3-*O*-methanesulphonate of 6-oxodec-4-en-9-olide (**5**) which was also prepared from cephalosporolide B by treatment with methanesulphonyl chloride. Treatment of cephalosporolide C (**4**) with methanesulphonyl chloride gave an unstable dimethanesulphonate (**6**) which on pyrolysis also afforded the 3-*O*-methanesulphonate of 6-oxodec-4-enolide (**5**).

Table 1. ^{13}C N.m.r. signals of the cephalosporolides B—F (determined in $CDCl_3$ at 25 MHz)

	δ/p.p.m.				
Carbon	(3)	(4)	(7)	(8)	(9)
1	168.6	160.1	172.8	175.6	175.4
2	43.3	44.1	39.7	41.4	42.0
3	64.0	69.6	69.7	77.1	76.9
4	138.5	75.1	38.3	83.1	83.6
5	132.5	46.8	21.5	37.3	36.7
6	207.7	200.0	34.8	114.9	115.3
7	38.5	42.8	76.1	34.0	35.8
8	32.3	37.8	19.0	31.1	32.2
9	71.3	72.3		74.8	76.5
10	18.9	25.0		20.7	22.6

Table 2. ^{1}H N.m.r. signals of the cephalosporolides B—F (determined in $CDCl_3$ at 220 MHz)

	δ/p.p.m.				
Proton	(3)	(4)	(7)	(8)	(9)
2	2.45	2.35	2.62	2.52	2.68
	2.95	2.93	2.95	2.67	2.73
3	5.22	4.25	4.15	4.82	4.75
4	5.8	3.45	1.75	5.09	5.05
5	6.25	2.75	n.a.*	2.02	2.27
				2.33	2.46
6			1.75		
7	n.a.*	n.a.*	4.75	1.99	2.05
8	2.05	2.05	1.35	1.33	1.67
				2.00	2.05
9	5.05	5.13		4.10	4.15
10	1.25	1.27		1.10	1.23

* n.a. = Not assigned.

Table 3. ^{1}H N.m.r. coupling constants of the cephalosporolides B—F

	J/Hz				
Protons	(3)	(4)	(7)	(8)	(9)
H_{2A}–H_{2B}	15	18	12	19	18
H_{2A}–H_3	3	12	6	1.5	0.5
H_{2B}–H_3	6	3	3.5	8	6
H_3–H_4	9	10		6	5
H_4–H_5	12				
H_4–H_{5A}		4		6	2
H_4–H_{5B}		4		0	7
H_{5A}–H_{5B}				14	15
H_{6A}–H_7			9		
H_{6B}–H_7			3		
H_7–H_8			6		
H_{8A}–H_9	7	7.5			
H_{8B}–H_9	4	5.0			
H_9–H_{10}	6.5	6.5		7	6

All three samples had identical i.r. and n.m.r. spectra, m.p., mixed m.p., and rotations. There are two suprising aspects of the inter-relationship. The first is the generation of the same compound on elimination of the C-4 epimers and the second is the stability of the 3-*O*-methanesulphonate. Examination of the *X*-ray structures of thiobiscephalosporolide A and of cephalosporolide C reveals that the epimeric hydrogen atoms at C-5 can come sufficiently close to the sulphoxide in one case and the methanesulphonate in the other to permit a *syn* elimination and thus afford the *cis*-olefin. The *X*-ray structure

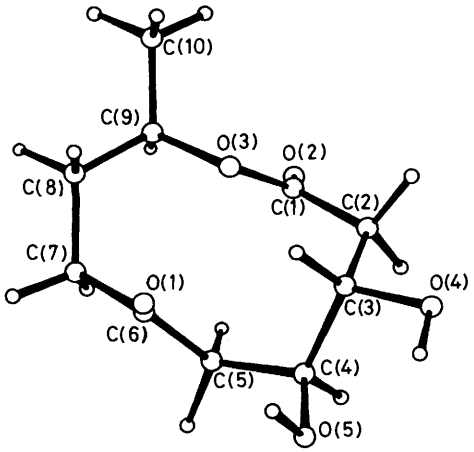


Figure 2. Structure of cephalosporolide C (**4**)

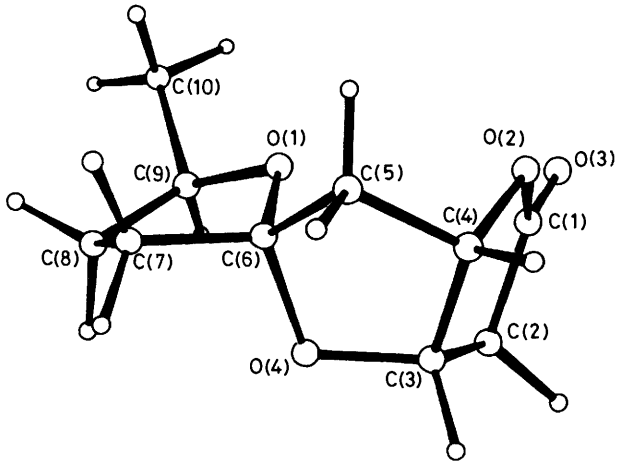
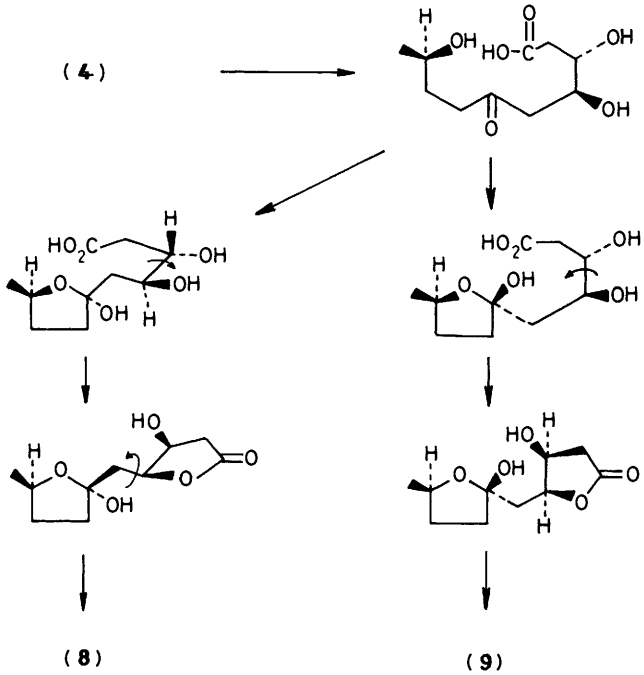


Figure 3. Structure of cephalosporolide E (**8**)

of the methanesulphonate also suggests that the stability of the 3-methylsulphonoxy group may lie in its preference for a conformation in which the methanesulphonate is too far from the hydrogen at C-2 for thermal *syn* elimination to occur.

Cephalosporolide D, $C_8H_{14}O_3$, M^+ 158 (**7**) showed i.r. absorption at 3 420 (OH) and 1 690 cm^{-1}. Unlike the other cephalosporolides, the ^{13}C n.m.r. spectrum possessed only eight signals which were assigned to a methyl group, four methylene groups, two CHO groups, and one CO·O group. ^1H N.m.r. spin decoupling studies showed that the methyl group doublet [δ 1.48 (*J* 6.5 Hz)] was coupled to a C*H*O·CO signal at δ 4.75. The spectrum also contained two double doublets at δ 2.65 (*J* 5.5 and 12.5 Hz) and δ 2.97 (*J* 4.5 and 12.5 Hz) which were geminally coupled (*J* 12.5 Hz). They were also coupled to a CHOH resonance at δ 4.15. This led to the structure 3-hydroxyoctan-7-olide (**7**) for the metabolite. Application of Horeau's method[7] to the determination of the absolute stereochemistry of the alcohol showed that it had the '*S*' configuration.

Two further isomeric $C_{10}H_{14}O_4$ lactones, cephalosporolides E (**8**) and F (**9**) were isolated from another fermentation of *C. aphidicola* grown under sulphur limiting conditions. Unlike the previous lactones they showed a single carbonyl absorption at 1 770—1 780 cm^{-1} (γ-lactone) and no hydroxy absorption. Their ^{13}C n.m.r. spectra (see Table 1) contained ten signals including singlets at δ 114.8 (E) and 115.3 (F) which were assigned to an acetal carbon. In addition there were three –CH·O– signals, four methylenes, and a methyl group. Bearing in mind the lack of hydroxy absorption in the i.r. spectrum, the ^{13}C n.m.r. spectra require the presence of two cyclic ethers and a γ-lactone ring. Whilst the ^1H n.m.r. spectra bore some similarities to the other cephalosporolides there were some significant differences. In the case of cephalosporolide E, irradiation at δ 4.07 rather than at a lower field signal led to collapse of the methyl group doublet [δ 1.1 (*J* 7 Hz)] to a singlet and caused perturbations to signals at δ 1.36 and 2.00 indicating the presence of the system (**12**). Irradiation of the resonance at δ 4.82 collapsed a triplet at δ 5.09 (*J* 6 Hz) to a broad doublet, removed an 8 Hz coupling from a double doublet (*J* 8 and 19 Hz) at δ 2.67 and a 1.5 Hz coupling from a second double doublet (*J* 1.5 and 19 Hz) at δ 2.52. Irradiation at δ 5.09 collapsed the signal at δ 4.82 to a broad doublet (*J* 8 Hz), removed a 6 Hz coupling from a double doublet (*J* 6 and 14 Hz) at δ 2.04, and sharpened a doublet (*J* 14 Hz) at δ 2.33. This data can be accommodated in part structure (**15**), leading to several plausible structures for the acetal. In view of the shortage of material, the final structure (**8**) but not the absolute configuration was elucidated by *X*-ray analysis, (see Figure 3). A comparable set of ^1H n.m.r. decoupling studies based on irradiation of the resonances at δ 1.23, 2.46, 4.16, 4.75, and 5.04 in cephalosporolide F suggest that it is the epimer (**9**).



Scheme. Formation of cephalosporolides E and F, (**8**) and (**9**)

Cephalosporolides E and F could arise by hydrolysis of cephalosporolide C, relactionization and acetal formation (see Scheme). However, attempts to mimic this in the laboratory were unsuccessful. Nevertheless, since these compounds were isolated only on one occasion after extensive chromatography, it is possible that they were artefacts of the isolation procedure.

Experimental

Isolation of the Cephalosporolides B—F.—A sample (50 g) of the crude residues obtained from a commercial fermentation of *Cephalosporium aphidicola* after the crystallization of aphidicolin, was chromatographed on silica (Merck, 7734, deactivated with 15% w/v water) (1 kg). Elution with 70% ethyl acetate–light petroleum gave a fraction which was purified by further

chromatography on silica and crystallization from ethyl acetate–light petroleum to afford *cephalosporolide* B (**3**) (200 mg) as needles, m.p. 119—122 °C, $[\alpha]_D^{20}$ +145° (*c* 5.4 in $CHCl_3$) (Found: C, 60.8; H, 6.85. $C_{10}H_{14}O_4$ requires C, 60.6; H, 7.12%), $\nu_{max.}$(Nujol) 3 320, 1 730, 1 690, and 1 640 cm^{-1}; $\lambda_{max.}$ *215 nm* (ε 5 080); *m/z* 198 (1%), 180 (5), 165 (1), 152 (5), 138 (50), 125 (40), 111 (43), and 55 (100). The ^1H and ^{13}C n.m.r. data are tabulated.

Elution with 90% ethyl acetate–light petroleum gave a fraction which was again purified by further chromatography on silica to afford *cephalosporolide* D (**7**) (300 mg) as needles, m.p. 130—132 °C, $[\alpha]_D^{20}$ −46.5° (*c* 2.23 in $CHCl_3$) (Found: C, 60.8; H, 8.9. $C_8H_{14}O_3$ requires C, 60.7; H, 8.9%); $\nu_{max.}$ 3 420, 1 690 cm^{-1}; *m/z* 158 (0.2%), 140 (1), 125 (0.8), 114 (13), and 42 (100).

Elution with ethyl acetate gave a third fraction which was purified by further chromatography and crystallization to afford *cephalosporolide* C (**4**) (500 mg), m.p. 93—96 °C, $[\alpha]_D^{20}$ +75° (*c* 1.7 in $CHCl_3$) (Found: C, 55.6; H, 7.43. $C_{10}H_{16}O_5$ requires C, 55.5; H, 7.46%); $\nu_{max.}$ 3 400, 1 725 cm^{-1}; *m/z* 216 (0.3%), 198 (0.6), 170 (5), 154 (8), 139 (10), 127 (95), 101 (52), 83 (90), and 55 (100).

On one occasion the residues (10 g) from *C. aphidicola* grown under sulphur limiting conditions were chromatographed on silica. Elution with 60% ethyl acetate–light petroleum gave *cephalosporolide* E (**8**) (590 mg) which crystallized as prisms, m.p. 101 °C, $[\alpha]_D^{30}$ +51.3° (*c* 0.42 in $CHCl_3$) (Found: C, 60.6; H, 7.3. $C_{10}H_{14}O_4$ requires C, 60.6; H, 7.12%), $\nu_{max.}$ 1 770 cm^{-1}; *m/z* 198 (8%), 183 (10), 154 (48), 143 (52), 139 (43), 127 (60), 111 (53), and 56 (100).

Elution with 2% methanol–ethyl acetate gave *cephalosporolide* F (**9**) (194 mg), m.p. 58—60 °C (Found: C, 60.6; H, 7.2. $C_{10}H_{14}O_4$ requires C, 60.6; H, 7.12%), $\nu_{max.}$ 1 780 cm^{-1}; *m/z* 198 (5%), 183 (7), 154 (27), 143 (43), 127 (55), 111 (50), and 56 (100).

Oxidation of Thiobiscephalosporolide A.—The bislactone (200 mg) in glacial acetic acid (10 ml) was treated with sodium metaperiodate (120 mg) in water (1 ml) at room temperature. After 90 min the solvents were evaporated and the product was chromatographed on silica to afford *4,4′-sulphinylbis(3-hydroxy-6-oxodecan-9-olide)* (**2**) (100 mg), m.p. 155—158 °C $[\alpha]_D^{25}$ −49° (*c* 0.24 in EtOH) (Found: C, 53.7; H, 6.4. $C_{20}H_{30}O_9S$ requires C, 53.8; H, 6.77%); $\nu_{max.}$ 3 340, 1 725, 1 708, and 1 050 cm^{-1}; δ($CHCl_3$) 1.25 (6 H, d, *J* 7 Hz), 3.57 (2 H, m, CHS), 4.68 (2 H, m, CHO), 5.07 (2 H, m, CHOCO). The *monomethanesulphonate*, prepared with methanesulphonyl chloride in pyridine, had m.p. 118—120 °C, $[\alpha]_D^{20}$ −41° (*c* 0.2 in $CHCl_3$) (Found: C, 48.4; H, 6.5. $C_{21}H_{32}O_{11}S_2$ requires C, 48.1; H, 6.15%); $\nu_{max.}$ 3 400br, 1 740, 1 710, and 1 050 cm^{-1}; δ 1.26 (6 H, m), 3.1 (3 H, s, $MeSO_3$), 4.65 (1 H, m, CHO), 5.1 (2 H, m, CHOCO), and 5.55 (1 H, m, $CHOSO_2Me$).

Pyrolysis of the Monomethanesulphonate.—The above methanesulphonate (900 mg) was heated at 130 °C for 20 min *in vacuo*. The residual black gum was chromatographed on silica in toluene–ethyl acetate (8:2) to afford the *3-methanesulphonate* (**5**) of cephalosporolide B (50 mg), m.p. 137—138 °C, $[\alpha]_D^{30}$ + 173° (*c* 0.25 in $CHCl_3$) (Found: C, 47.6; H, 6.0. $C_{11}H_{16}O_6S$ requires C, 47.8; H, 5.84%), $\nu_{max.}$ 1 745, 1 690, 1 640, 1 355, and 1 175 cm^{-1}; $\lambda_{max.}$ 219 nm (ε 5 000); δ 1.27 (3 H, d, *J* 6 Hz), 3.13 (3 H, s), 5.00 (1 H, m), 5.67, 5.80 and 6.63 (1 H each). The same (i.r., n.m.r., $[\alpha]_D$, and mixed m.p.) methanesulphonate was obtained from cephalosporolide B with methanesulphonyl chloride in pyridine.

Cephalosporolide C *bismethanesulphonate* (**6**). This was prepared with methanesulphonyl chloride in pyridine. It crystallized from ethyl acetate–light petroleum as needles, m.p. 124 °C, $[\alpha]_D^{30}$ +38° (*c* 0.2 in $CHCl_3$) (Found: C, 38.9; H, 5.4. $C_{12}H_{20}O_9S_2$ requires C, 38.7; H, 5.41%); $\nu_{max.}$ 1 735, 1 715, and 1 700 cm^{-1}; δ 1.3 (3 H, d, *J* 6.5 Hz), 3.1 and 3.17 (each 3 H, s, OSO_2Me), and 5.1 (3 H, m, CHOR).

Pyrolysis of the Bismethanesulphonate (**6**).—The bismethanesulphonate (200 mg) was heated at 120 °C *in vacuo* for 5 min. The residue was chromatographed on silica in toluene–ethyl acetate to afford the 3-methanesulphonate (**5**) of cephalosporolide B, m.p. 139—140 °C, $[\alpha]_D^{25}$ +173°, identical with the material described above (i.r., n.m.r., and mixed m.p.).

Crystal Structure Determinations.—(*a*) *Cephalosporolide* B 3-O-*methanesulphonate* (**5**). $C_{11}H_{16}O_6S$, M = 276.3, orthorhombic, a = 5.545(1), b = 11.272(1), c = 20.739(2) Å, U = 1 296.3 Å^3, Z = 4, D_c = 1.42 g cm^{-3}, $F(000)$ = 584. Monochromated Mo-K_α radiation, λ = 0.710 69 Å, μ = 2.7 cm^{-1}. Space group $P2_12_12_1$ from systematic absences of *h*00 for *h* odd, 0*K*0 for *k* odd, and 00*l* for *l* odd.

(*b*) *Cephalosporolide* C (**4**). $C_{10}H_{16}O_5$, M 216.24, monoclinic, a = 5.566(1), b = 7.759(2), c = 12.633(3) Å, β = 92.84(2)°, U = 544.9 Å^3, Z = 2, D_c = 1.32 g cm^{-3} $F(000)$ = 232. Monochromated Mo-K_α radiation λ = 0.710 69 Å, μ = 1.1 cm^{-1}. Space group $P2_1$ from systematic absences of 0*k*0 for *k* odd and successful refinement.

(*c*) *Cephalosporolide* E (**8**). $C_{10}H_{14}O_4$, M = 198.2, orthorhombic, a = 6.431, b = 9.967, c = 15.518 Å, U = 994.7 Å^3, Z = 4, D_c = 1.32 g cm^{-3}, $F(000)$ = 424. Monochromated Mo-K_α radiation, λ = 0.710 69 Å, μ = 1.1 cm^{-1}. Space group $P2_12_12_1$ from systematic absences of *h*00 for *h* odd, 0*k*0 for *k* odd, and 00*l* for *l* odd.

In each case data were measured on an Enraf-Nonius CAD4 diffractometer using a crystal of *ca.* 0.25 × 0.25 × 0.25 mm. Preliminary cell dimensions were found using the SEARCH and INDEX routines of the CAD4 and final values were calculated from the setting angles for 25 reflections with θ *ca.* 15°. Intensities for *hkl* reflections with $2 < \theta < 25°$ were measured by a θ/2θ scan with a scan width of $\Delta\theta = (1.0 + 0.35\tan\theta)°$. The scan rate for each reflection was determined by a rapid prescan at 10° min^{-1} in θ at which point any reflection with $I < \sigma(I)$ was coded as unobserved. The remaining reflections were rescanned at such a speed as to give a minimum value of $\sigma(I)/I$ of 0.05 subject to a maximum scan time of 120 s. Two standard reflections monitored every hour showed no significant variation. Data were corrected for Lorentz polarization (Lp) effects but not for absorption and after averaging any equivalent reflections 1 125 (cephalosporolide B 3-*O*-methanesulphonate), 789 (cephalosporolide C), and 686 (cephalosporolide E) reflections with $|F^2| > \sigma(F^2)$ were used in the structure refinement. The values of $\sigma(F^2)$ were taken as $[\sigma^2(I) + (0.031)^2]^{\frac{1}{2}}/\text{Lp}$. The structures were solved by direct methods using the MULTAN program. Refinement of non-hydrogen atoms with anisotropic temperature factors was by full-matrix least-squares. Hydrogen atoms were placed at calculated positions (C–H 1.08 Å) and held fixed with a common isotropic temperature factor of B = 5.0 Å^2. Refinement converged at R = 0.055, R' = 0.082 when the maximum shift/error was 0.08 (cephalosporolide B 3-*O*-methanesulphonate), R = 0.051, R' = 0.057 when the maximum shift/error was 0.01 (cephalosporolide C), R = 0.047, R' = 0.056 when the maximum shift/error was 0.01 (cephalosporolide E). The weighting schemes were $w = 1/\sigma^2(F)$. The final difference maps were everywhere featureless.

The structure solutions and refinement were done on a PDP 11/34 computer using the Enraf-Nonius Structure Determination Package. Scattering factors were taken from the International Tables for *X*-Ray Crystallography, 1974, vol. 4. Final atomic co-ordinates are listed in Tables 4—6. Lists of temperature factors and hydrogen atom positions have been deposited

Table 4. Fractional atomic co-ordinates (× 10^4) of cephalosporolide B 3-*O*-methanesulphonate (**5**) with e.s.d.s. in parentheses

	x	*y*	*z*
S	1 487.0(27)	4 758.6(13)	6 358.9(6)
O(1)	1 547(10)	7 059(4)	7 980(2)
O(2)	2 841(13)	3 035(4)	9 090(2)
O(3)	981(7)	4 680(3)	8 762(2)
O(4)	2 949(8)	4 071(3)	6 890(2)
O(5)	−893(8)	4 971(5)	6 573(2)
O(6)	1 869(10)	4 072(4)	5 782(2)
C(1)	2 124(12)	3 650(5)	8 659(3)
C(2)	2 361(14)	3 363(5)	7 973(2)
C(3)	2 745(11)	4 485(5)	7 564(2)
C(4)	5 080(10)	5 085(6)	7 742(3)
C(5)	5 253(12)	6 059(6)	8 104(3)
C(6)	3 142(13)	6 721(5)	8 335(3)
C(7)	3 025(14)	6 971(5)	9 035(3)
C(8)	779(12)	6 466(6)	9 371(3)
C(9)	771(12)	5 118(5)	9 440(2)
C(10)	−1 454(15)	4 627(7)	9 743(3)
C(11)	3 016(17)	6 115(6)	6 307(4)

Table 5. Fractional atomic co-ordinates (× 10^4) of cephalosporolide C (**4**) with e.s.d.s in parentheses

	x	*y*	*z*
O(1)	7 742(5)	3 301	1 427(2)
O(2)	5 737(8)	8 494(6)	3 591(3)
O(3)	7 862(5)	6 153(5)	3 220(2)
O(4)	9 879(5)	8 255(5)	441(2)
O(5)	6 801(5)	5 906(5)	−394(2)
C(1)	7 072(9)	7 761(7)	3 039(4)
C(2)	8 113(9)	8 523(7)	2 075(4)
C(3)	8 551(8)	7 283(6)	1 175(3)
C(4)	6 249(8)	6 645(6)	594(3)
C(5)	4 713(7)	5 457(6)	1 252(3)
C(6)	5 944(8)	3 937(6)	1 775(3)
C(7)	4 841(9)	3 186(7)	2 731(4)
C(8)	6 538(10)	3 332(9)	3 691(4)
C(9)	6 864(10)	5 190(8)	4 097(3)
C(10)	8 599(12)	5 291(11)	5 011(5)

* For details of the Supplementary Publications Scheme see Instructions for Authors (1985) in *J. Chem. Soc., Perkin Trans. 1*, 1985, Issue 1.

Table 6. Fractional atomic co-ordinates (× 10^4) of cephalosporolide E (**8**) with e.s.d.s in parentheses

	x	*y*	*z*
O(1)	4 382(5)	4 669(3)	6 146(2)
O(2)	3 541(5)	3 723(3)	7 934(2)
O(3)	6 945(6)	3 459(4)	8 076(3)
O(4)	2 716(6)	2 608(3)	6 000(2)
C(1)	5 334(8)	3 072(5)	7 775(3)
C(2)	4 944(10)	1 901(5)	7 197(3)
C(3)	2 766(9)	2 099(4)	6 866(3)
C(4)	1 855(7)	3 200(5)	7 434(3)
C(5)	1 089(7)	4 247(5)	6 801(3)
C(6)	2 425(7)	4 030(4)	6 021(3)
C(7)	1 722(8)	4 482(5)	5 142(3)
C(8)	3 722(9)	4 554(6)	4 643(3)
C(9)	5 398(7)	4 835(5)	5 321(3)
C(10)	6 340(11)	6 205(6)	5 299(4)

as a Supplementary Publication (SUP No. 56165, 13 pp.).* Final structure factors are available on request from the editorial office.

References

1 W. Dalziel, B. Hesp, K. M. Stevenson, and J. A. J. Jarvis, *J. Chem. Soc., Perkin Trans. 1*, 1973, 2841.
2 M. J. Ackland, J. R. Hanson, A. H. Ratcliffe, and I. H. Sadler, *J. Chem. Soc., Chem. Commun.*, 1982, 165.
3 R. P. Mabelis, A. H. Ratcliffe, M. J. Ackland, J. R. Hanson, and P. B. Hitchcock, *J. Chem. Soc., Chem. Commun.*, 1981, 1006.
4 T. Ishida and K. Wada, *J. Chem. Soc., Chem. Commun.*, 1975, 209; K. Wada and T. Ishida, *J. Chem. Soc., Chem. Commun.*, 1976, 340; *J. Chem. Soc., Perkin Trans. 1*, 1979, 1154.
5 M. Nukina, T. Sassa, and M. Ikeda, *Tetrahedron Lett.*, 1980, 301; *Agric. Biol. Chem. (Japan)*, 1980, **44**, 2761.
6 L. M. Jackman and S. Sternhell, 'Applications of NMR Spectroscopy in Organic Chemistry,' Pergamon Press, Oxford, 1969, p. 302.
7 A. Horeau and A. Nouaille, *Tetrahedron Lett.*, 1971, 1939.

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