The Structure of Thiobiscephalosporolide A, A Dimeric Pentaketide Macrolide from *Cephalosporium aphidicola*

Mark J. Ackland, James R. Hanson,* and Peter B. Hitchcock
School of Molecular Sciences, University of Sussex, Brighton, Sussex BN1 9QJ
Richard P. Mabelis and Arnold H. Ratcliffe
I.C.I. Pharmaceuticals Division, P. O. Box 25, Mereside, Alderley Park, Macclesfield, Cheshire SK10 4TG

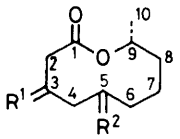
The isolation, structure elucidation, and some chemical transformations of the dimeric macrolide, thiobiscephalosporolide A, are reported.

Recently a number of pentaketide fungal metabolites containing ten-membered lactone rings have been isolated. These include the diplodialides A—D (**1**)—(**4**),[1-3] obtained from *Diplodia pinea*, and the pyrenolides A—C (**5**)—(**7**)[4,5] which were isolated from *Pyrenophora teres*. A hexaketide twelve-membered lactone, recifeiolide (**8**)[6] has been obtained from *Cephalosporium recifei* whilst cladospolide A (**9**)[7] is a phytotoxic metabolite of *Cladosporium fulvum*. Because of their biological activity and synthetic challenge they pose, there is considerable interest in these compounds[8-15] and the related phorocantholides,[16] which are produced by insects. In a preliminary communication[17] we described a dimeric lactone, thiobiscephalosporolide A (**10**), obtained from *Cephalosporium aphidicola*. In this paper we present our full evidence for the structure together with some related chemical transformations.

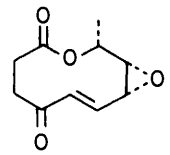
Thiobiscephalosporolide A, $C_{20}H_{30}O_8S$ (M^+ 430) (**10**), showed i.r. absorptions at 3 500 cm^{-1} (OH) and 1 725 and 1 705 cm^{-1} (C=O). In its mass spectrum it showed the loss of two molecules of water (430→412→394) and two molecules of carbon monoxide (412→384→356) confirming the presence of hydroxy and carbonyl groups. The ^1H n.m.r. spectrum (see Table 1) contained resonances attributable to two secondary methyl groups [δ 1.18 (6 H, d, *J* 7.2 Hz)], a secondary thioether [δ 3.95 (2 H, m)], two secondary alcohols [δ 4.7 (2 H, m)], and two secondary lactones [δ 5.02 (2 H, m)]. However the ^{13}C n.m.r. spectrum (Table 2) contained only ten signals (1 Me, 4 CH_2, 3 CHX, 1 O.CO and 1 CO) suggesting that the molecule was a C_{10} dimer.

Thiobiscephalosporolide A formed a diacetate, $C_{24}H_{34}O_{10}S$ (M^+ 514) (**11**), and a bis(trimethylsilyl) ether, and was oxidized to a diketone, $C_{20}H_{26}O_8S$ (M^+ 426) (**12**), with pyridinium dichromate. These compounds did not show a hydroxy absorption in their spectra and hence thiobiscephalosporolide A contains only two hydroxy groups. Reduction with Raney nickel gave a monomeric desthio compound, cephalosporolide A, $C_{10}H_{16}O_4$ (M^+ 200) (**13**). This compound showed i.r. absorptions at 3 400 (OH), 1 730 (O.CO), and 1 680 (CO) cm^{-1}, and ^{13}C n.m.r. signals (Table 2) attributable to 1 Me, 5 CH_2, 2 CHX, a CO_2 and a CO group, suggesting that thiobiscephalosporolide A consists of two monomeric cephalosporolide A units joined by a sulphur bridge.

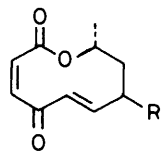
Extensive ^1H n.m.r. spin decoupling studies at 360 MHz on thiobiscephalosporolide and its derivatives enabled several part structures to be deduced. Thus irradiation at δ 5.02 led to the collapse of the methyl group doublet (δ 1.18) to a singlet and perturbed a four-proton multiplet at δ 2.0, suggesting the presence of the part structure $CH_2CH(O.CO)Me$. Irradiation at δ 4.70 resulted in the collapse of the multiplet at δ 3.95 to a double doublet and the conversion of the signals at δ 2.80 and 2.60 into a geminally coupled AB doublet, *J* 16.5 Hz. Irradiation at δ 3.95 not only confirmed the coupling to the signal at δ 4.70 but also reduced the two signals at δ 2.70 and 2.98 to an AB doublet with *J* 18 Hz. These results can be accommodated by the part structure $OCCH_2CH(OH)CH(S)CH_2CO$. The presence of this partial structure was confirmed by a comparable series of spin-decoupling experiments on the acetate (**11**) in which the resonance attributed to the secondary alcohol had moved



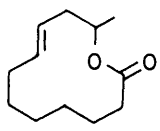
(**1**) R^1 = O, R^2 = H_2
(**2**) R^1 = H,OH, $\Delta^{4,5}$; R^2 = H
(**3**) R^1 = H,OH , R^2 = H_2
(**4**) R^1 = H,OH , R^2 = O



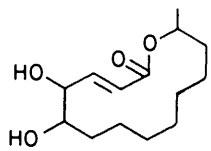
(**5**)



(**6**) R = H
(**7**) R = OH



(**8**)



(**9**)

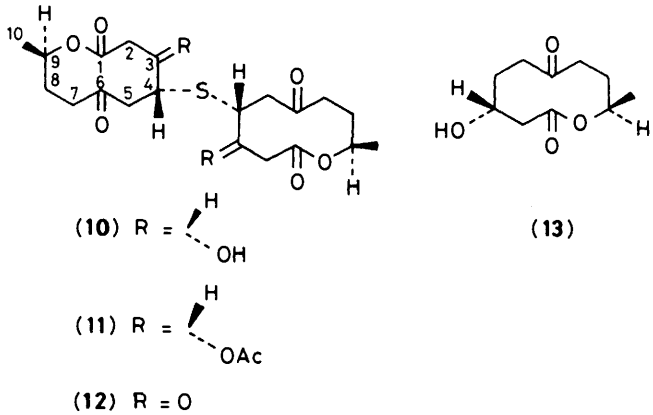
Table 1. ^1H N.m.r. signals of thiobiscephalosporolide A and its derivatives (determined in [$^2H_5$]pyridine at 360 MHz)

	δ/p.p.m.			
H	(**10**)	(**11**)	(**12**)	(**13**)
2	2.60	2.85	3.57	2.50
	2.86	3.25	4.60	2.95
3	4.70	6.02		4.48
4	3.95	4.38	4.95	*a*
5	2.70	2.80	2.90	*a*
	2.98	2.98	3.30	
7	2.25	2.30	2.30	*a*
8	1.95	1.8	1.90	1.6
9	5.02	5.05	5.1	5.05
10	1.18	1.05	1.05	1.17

[a] Not assigned.

Table 2. ^{13}C N.m.r. signals of thiobiscephalosporolide A and its derivatives (determined in [$^2H_5$]pyridine)

Carbon atom	δ/p.p.m. (10)	(11)	(12)	(13)
1	170.1	169.5	165.6	170.4
2	40.2	35.3	44.7	40.2
3	66.7	69.8	195.9	64.8
4	50.8	45.9	50.7	31.9
5	47.4	46.2	44.7	42.5
6	208.7	206.9	205.3	210.9
7	39.5	39.9	39.4	38.8
8	33.9	33.9	33.2	33.7
9	71.9	72.6	73.4	72.0
10	19.5	19.5	19.5	19.6
OAc		21.1, 168.5		



(10) R = H, OH

(11) R = H, OAc

(12) R = O

(13)

downfield to δ 6.02. The diketone (**12**) contained an isolated AB system [δ 3.57 and 4.60 (J 16.1 Hz)] and a clearly resolved ABX system [δ 2.9, 3.3, and 4.95 (J_{AB} 16.7 Hz; J_{AX} 12.8 Hz; J_{BX} 3.3 Hz)]. These partial structures are best accommodated by the decanolide structure (**10**). However, it was not possible to relate the stereochemistry of the lactone with that of the vicinal thioalcohol and hence the overall structure and relative configuration were established by *X*-ray analysis.

The ^{13}C n.m.r. spectra of thiobiscephalosporolide A and its derivatives were readily assigned on the basis of known substituent effects. Thus the resonances attributed to the methyl group, the carbonyl, hydroxy, and thioether carbon atoms were within the expected regions. The signals assigned to the 7- and 8-methylenes were distinguished from those at C-2 and C-5 by the changes in the latter on acetylation and oxidation of thiobiscephalosporolide A.

The absolute configuration of the metabolite was then determined by applying Horeau's method [18] to the alcohol. This established that the hydroxy group possessed the *S* configuration and hence the overall absolute stereochemistry is as shown in structure (**10**).

Thiobiscephalosporolide A is unusual in that it is the first dimeric thiomacrolide to be isolated; furthermore the position of the ketone does not correspond to an acetate carboxy group in the pentaketide chain, and the diplodialides, recifeiolide, and thiobiscephalosporolide A all possess the *R* configuration at the terminus of the lactone ring. There is some irony in the fact that the other major metabolite of this strain of *Cephalosporium aphidicola* is also C_{20} but is a diterpenoid, formed by a completely different biosynthetic pathway.

Experimental

Isolation of Thiobiscephalosporolide A (**10**).—The crude ethyl acetate extract of a large scale industrial fermentation of *Cephalosporium aphidicola* (ACC 3490) was repeatedly crystallized to remove aphidicolin. The third crop, a very dark, brown solid, contained significant quantities of thiobiscephalosporolide A as determined by t.l.c. A sample of this material (1 g) was purified by dissolution in hot methanol (25 ml) containing decolourizing charcoal. After filtration and concentration to a small volume (*ca.* 5 ml), the solid was allowed to crystallize at room temperature. The product (450 mg) still contained some aphidicolin and was recrystallized a second time to afford *thiobiscephalosporolide A* (350 mg) representing 3 mg l^{-1} of the original fermentation. The lactone (**10**) crystallized as rhombs, m.p. 210—213 °C, $[\alpha]_D^{30}$ −65.3° (*c* 7 in $CHCl_3$) (Found: C, 55.9; H, 7.0. $C_{20}H_{30}O_8S$ requires C, 55.8; H, 7.02%); $\nu_{max.}$ 3 500, 1 725, and 1 705 cm^{-1}; *m/z* 412 (10%), 384 (5), 324 (2), 125 (30), 111 (70), and 55 (100). The ^1H and ^{13}C n.m.r. spectra are given in Tables 1 and 2. The *diacetate* (**11**), prepared with acetic anhydride in pyridine, had m.p. 206—209 °C, $[\alpha]_D^{32}$ +39° (*c* 1 in $CHCl_3$) (Found, C, 56.0; H, 6.5. $C_{24}H_{34}O_{10}S$ requires C, 56.0; H, 6.6%); $\nu_{max.}$ 1 730 cm^{-1}; *m/z* 514 (0.1%), 454 (35), 394 (40), 366 (8), 333 (70, and 43 (100).

Oxidation of Thiobiscephalosporolide A (**10**).—Thiobiscephalosporolide A (100 mg) in dimethylformamide (5 ml) was treated with pyridinium dichromate (606 mg) for 6 h at room temperature. The solution was poured into water and the product was recovered in ethyl acetate. *Thiobisdioxocephalosporolide A* (**12**) (77 mg) crystallized from ethyl acetate–light petroleum as needles, m.p. 171—174 °C, $[\alpha]_D^{32}$ −66° (*c* 4.8 in $CHCl_3$) (Found: C, 56.0, H, 5.6. $C_{20}H_{26}O_8S$ requires C, 56.3; H, 6.2%), $\nu_{max.}$ 1 740, 1 710 cm^{-1}; *m/z* 426 (3%), 339 (3), 256 (13), 230 (25), and 101 (100).

Reduction of Thiobiscephalosporolide A (**10**) *with Raney Nickel.*—W-2 Raney nickel (12 g) in ethanol (60 ml) was refluxed for 2 h with acetone (10 ml). Thiobiscephalosporolide A (200 mg) was added and the reaction mixture was refluxed for a further 4 h. The solution was filtered and the solvents evaporated to give a gum. This was purified by preparative layer chromatography on silica in chloroform–methanol (1:1) to afford *cephalosporolide A* (**13**) (94 mg) which crystallized from ethyl acetate–light petroleum as needles, m.p. 79 °C, $[\alpha]_D^{20}$ − 17.8° (*c* 0.5 in $CHCl_3$) (Found: C, 60.0; H, 8.05. $C_{10}H_{16}O_4$ requires C, 60.0, H, 8.06%); $\nu_{max.}$ 3 400, 1 730, and 1 685 cm^{-1}; *m/z* 200 (1%), 177 (30), 170 (28), 154 (34), 98 (95), and 43 (100).

Absolute Configuration of Thiobiscephalosporolide A (**10**).—Thiobiscephalosporolide A (430.5 mg) and 2-phenylbutyric anhydride (0.6 ml) were dissolved in pyridine (5 ml) and the optical rotation was measured during 3 h at room temperature until stable. Water (0.25 ml) was added and the optical rotation (α_1 +3.215°) noted after 30 min. Triethylamine (0.5 ml) was added and a second optical rotation (α_2 +4.043) measured immediately. Using the formula $\alpha_1 - 1.1\alpha_2$, the change in rotation was −1.232° indicating that the alcohol possessed the *S* configuration. Reference samples of menthol (*R*) and testosterone (*S*) were run as controls and gave the correct result.

X-*Ray Crystallographic Data.*—The crystal structure data were deposited with the Cambridge Crystallographic Data Centre, University of Cambridge, Lensfield Road, Cambridge CB2 1EW at the time of the preliminary communication.

Acknowledgements

We thank the S.E.R.C. and I.C.I. for a CASE award studentship for M. J. A.

References

1 T. Ishida and K. Wada, *J. Chem. Soc., Chem. Commun.*, 1975, 209.
2 K. Wada and T. Ishida, *J. Chem. Soc., Chem. Commun.*, 1976, 340.
3 K. Wada and T. Ishida, *J. Chem. Soc., Perkin Trans. 1*, 1979, 1154.
4 M. Nukina, T. Sassa, and M. Ikeda, *Tetrahedron Lett.*, 1980, 301.
5 M. Nukina, M. Ikeda, and T. Sassa, *Agric. Biol. Chem.*, 1980, **44**, 2761.
6 R. F. Vesonder, F. H. Stodola, L. J. Wickerham, J. J. Ellis, and W. K. Rohwedder, *Can. J. Chem.*, 1971, **49**, 2029; H. Gerlach, K. Oertle, and A. Thalmann, *Helv. Chim. Acta*, 1976, **59**, 755.
7 A. Hirota, A. Isogai, and H. Sakai, *Agric. Biol. Chem.*, 1981, **45**, 799.
8 T. Ishida and K. Wada, *J. Chem. Soc., Perkin Trans. 1*, 1979, 323.
9 A. B. Shenviaand and H. Gerlach, *Helv. Chim. Acta*, 1980, **63**, 2426.
10 T. Wakamatsu, K. Akasaka, and Y. Ban, *J. Org. Chem.*, 1979, **44**, 2008.
11 J. R. Mahajan and I. S. Resck, *Synthesis*, 1980, **12**, 998.
12 J. Tsuji and T. Mandai, *Tetrahedron Lett.*, 1978, 1817.
13 M. Petrzilka, *Helv. Chim. Acta*, 1978, **61**, 3075.
14 R. Malherbe and D. Bellus, *Helv. Chim. Acta*, 1978, **61**, 3096.
15 R. E. Ireland and F. R. Brown, *J. Org. Chem.*, 1980, **45**, 1868.
16 B. P. Moore and W. Vance Brown, *Aust. J. Chem.*, 1976, **29**, 1365.
17 R. P. Mabelis, A. H. Ratcliffe, M. J. Ackland, J. R. Hanson, and P. B. Hitchcock, *J. Chem. Soc., Chem. Commun.*, 1981, 1006.
18 A. Horeau and A. Nouaille, *Tetrahedron Lett.*, 1971, 1939.

Received 14*th March* 1984; *Paper* 4/410