Sirodesmins A, B, C, and G, Antiviral Epipolythiopiperazine-2,5-diones of Fungal Origin: *X*-Ray Analysis of Sirodesmin A Diacetate

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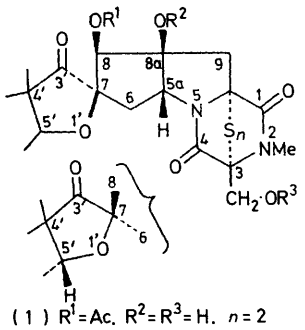
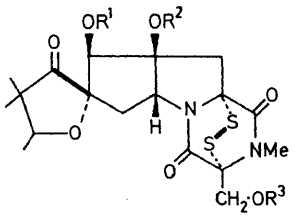
The isolation, structures, and absolute configurations of sirodesmins A, B, C, and G, epipolythiopiperazine-2,5-dione antibiotics produced by *Sirodesmium diversum*, are reported. The structure of sirodesmin A (1), an epidithiopiperazinedione, was determined by *X*-ray analysis of the derived diacetate (6). The structures of sirodesmins B (3), C (2), and G (7), epi-tetra-, -tri-, and -di-thiopiperazinediones, respectively, were determined by comparison of their chemical and spectroscopic properties with those of sirodesmin A. Sirodesmins B and C differ from sirodesmin A only in the size of the polysulphide bridge; sirodesmins A and G are epimers.

THE epipolythiopiperazinedione group is the common structural feature of an important class of fungal metabolites which includes gliotoxin,[1] the sporidesmins,[2] the aranotins,[3-6] the chaetocins [7,8] (verticillins [9]), chaeto-

[1] For references see S. Wilkinson and J. F. Spilsbury, *Nature*, 1965, **206**, 619.
[2] For example, E. Francis, R. Rahman, S. Safe, and A. Taylor, *J.C.S. Perkin I*, 1972, 470.
[3] N. Neuss, R. Nagarajan, B. B. Molloy, and L. L. Huckstep, *Tetrahedron Letters*, 1968, 4467.
[4] R. Nagarajan, L. L. Huckstep, D. H. Lively, D. C. DeLong, M. M. Marsh, and N. Neuss, *J. Amer. Chem. Soc.*, 1968, **90**, 2980.
[5] D. B. Cosulich, N. R. Nelson, and J. H. van der Hende, *J. Amer. Chem. Soc.*, 1968, **90**, 6519.
[6] P. A. Miller, P. W. Trown, W. Fulmor, G. O. Morton, and J. Karliner, *Biochem. Biophys. Res. Comm.*, 1968, **33**, 219.
[7] D. Hauser, H. P. Weber, and H. P. Sigg, *Helv. Chim. Acta*, 1970, **53**, 1061.
[8] D. Hauser, H. R. Loosli, and P. Niklaus, *Helv. Chim. Acta*, 1972, **55**, 2182.
[9] H. Minato, M. Matsumoto, and T. Katayama, *J.C.S. Perkin I*, 1973, 1819.

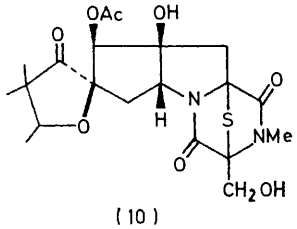
min,[10,11] the hyalodendrins,[12-16] the melinacidins,[17] and possibly oryzachlorin.[18] We report here the isolation





(1) R^1=Ac. R^2=R^3=H. n=2
(2) R^1=Ac. R^2=R^3=H. n=3
(3) R^1=Ac. R^2=R^3=H. n=4
(4) R^1=R^2=R^3=H. n=2
(5) R^1=R^3=Ac. R^2=H. n=2
(6) R^1=R^2=R^3=Ac. n=2

(7) R^1=Ac. R^2=R^3=H
(8) R^1=R^2=R^3=H
(9) R^1=R^2=R^3=Ac



(10)

of four new members of this class for which we propose the names sirodesmins A, B, C, and G and to which we assign structures (1), (3), (2), and (7), respectively. Sirodesmins A, B, and C are the major metabolites and sirodesmin G is one of at least five minor metabolites produced by the fungus *Sirodesmium diversum*.[19] Each of the sirodesmins may be obtained as an amorphous solid by a lengthy purification procedure involving solvent extraction, liquid–liquid partition, and column and thin-layer chromatography.

The spectroscopic, analytical, and chemical properties of the sirodesmins were in each case consistent with the presence of an epipolythiopiperazinedione substructure. Sirodesmins A, B, and C were found to contain two, four, and three sulphur atoms, respectively, and clearly differed only in the size of the polysulphide bridge. Thus the mass spectra of sirodesmins A, B, and C were virtually identical, the peak of highest *m/e* value in each case corresponding to the didethio fragment, $C_{20}H_{26}N_2O_8$. Also, sirodesmin A was readily converted into a mixture of sirodesmins B and C on treatment with sulphur in pyridine and, whereas solutions of sirodesmin A in ethanol or pyridine were stable, similar solutions of sirodesmins B or C were converted spontaneously into mixtures of sirodesmins A, B, and C.

Although several structural features of the sirodesmins were apparent from spectroscopic studies it was evident that a protracted investigation would be required to determine the structures of the metabolites by degradative procedures; accordingly we resorted to *X*-ray methods. This resulted in the structure of sirodesmin A being established as (1) by *X*-ray analysis of the fully acetylated derivative (6). By inference, the structures of sirodesmins B and C are (3) and (2), respectively. Di-, tri-, and tetra-thio-analogues have also been observed in the sporidesmin and hyalodendrin series and in the former series the three analogues were isolated from cultures of the same organism.

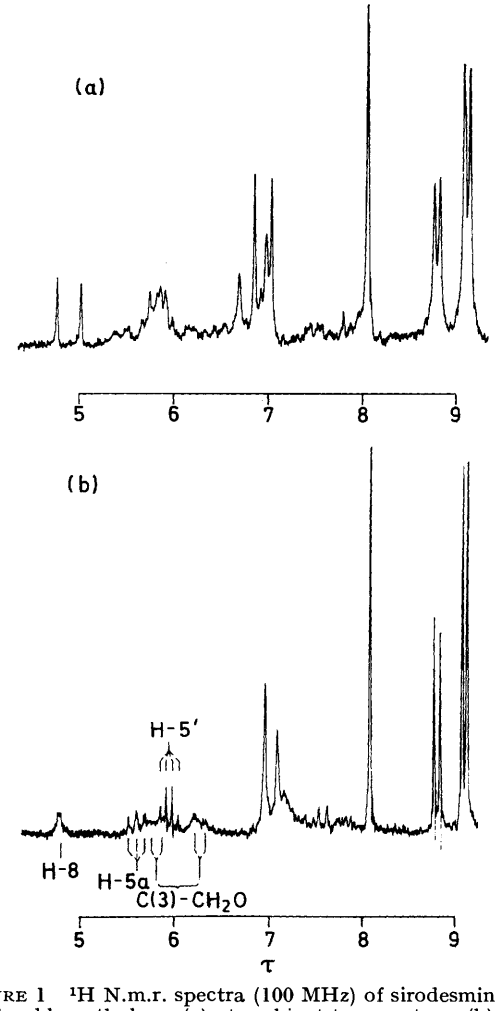


FIGURE 1 ^{1}H N.m.r. spectra (100 MHz) of sirodesmin C (2) in tetrachloroethylene, (a) at ambient temperature, (b) at 120°

The ^{1}H n.m.r. spectra of sirodesmins A and B and a number of derivatives are given in Table 1. Conversion

[10] S. Safe and A. Taylor, *J.C.S. Perkin I*, 1972, 472.
[11] S. Safe, A. Taylor, L. C. Vining, R. McG. Archibald, R. G. Stevenson, C. J. Mirocha, and C. M. Christensen, *Canad. J. Microbiol.*, 1972, **18**, 1129.
[12] G. M. Strunz, M. Kakushima, M. A. Stillwell, and C. J. Heisner, *J.C.S. Perkin I*, 1973, 2600.
[13] M. A. Stillwell, L. P. Magasi, and G. M. Strunz, *Canad. J. Microbiol.*, 1974, **20**, 759.
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[16] K. H. Michel, M. O. Chaney, N. D. Jones, M. M. Hoehn, and R. Nagarajan, *J. Antibiotics (Japan)*, 1974, **27**, 57.
[17] A. D. Argoudelis, *J. Antibiotics (Japan)*, 1972, **25**, 171.
[18] A. Kato, T. Saeki, S. Suzuki, K. Ando, G. Tamura, and K. Arima, *J. Antibiotics (Japan)*, 1969, **22**, 322.
[19] B.P. 1,387,504.

of the sirodesmins into the corresponding urethanes, by treatment with trichloracetyl isocyanate, proved a convenient structural probe for ^{1}H n.m.r. studies. The ^{1}H n.m.r. spectra of sirodesmin C (Figure 1) and the derived bisurethane in a variety of solvents were complex and, although they were similar in some respects to the spectra of sirodesmins A and B, some of the signals appeared to be duplicated, consistent with the presence of different conformations of the trisulphide group. In the case of the trisulphide sporidesmin E, where a similar phenomenon was found to occur,[20] many of the duplicated signals collapsed to single bands at 95 °C. A similar simplification of the spectrum of sirodesmin C in trichloroethylene was observed at 120 °C. The change was reversible.

The absolute configuration of gliotoxin and related epidithiopiperazinediones may be determined from the sign of the charge-transfer bands at *ca.* 270 and 310—320 nm in their c.d. spectra.[21] The c.d. spectrum of sirodesmin A in methanol ($\Delta\varepsilon_{310}$ — 0.99, $\Delta\varepsilon_{263}$ + 3.59, $\Delta\varepsilon_{235}$ — 32.20) is very similar to those of gliotoxin and aranotin and hence the absolute configurations of sirodesmins A, B, C, and gliotoxin are the same and are as depicted in the structures.

Sirodesmin G is isomeric with sirodesmin A. The mass spectra and ^{13}C n.m.r. spectra (Table 2) of the two metabolites were very similar. The resemblance between the ^{1}H n.m.r. spectra of the two metabolites and those of a number of their derivatives (Table 1) was particularly striking, since corresponding signals, differing only by small changes in chemical shift and coupling constants, were observed in each pair of spectra. In view of these similarities it is unlikely that the basic ring systems are grossly different in sirodesmins A and G.

Possible differences between the metabolites are: (*a*) the disulphide bridge may be on opposite faces of the piperazinedione ring, (*b*) substituents at carbon atoms 4′ and 5′ may be interchanged, and (*c*) one or more of the centres 5a, 7, 8, 8a, and 5′ may be of opposite chirality. Case (*a*) may be dismissed, since the chirality of the disulphide groups is the same in each metabolite, as was evident from the close resemblance between the c.d. spectra of sirodesmins A (see above) and G in methanol ($\Delta\varepsilon_{310}$ — 0.29, $\Delta\varepsilon_{263}$ + 8.4, $\Delta\varepsilon_{235}$ — 30.94). Case (*b*) is incompatible with the furan methine proton resonance

TABLE 1

Chemical shifts (τ values) for protons in sirodesmins A, B, and G and derivatives [a]

Compound	C(8)H (*J*/Hz)	C(5a)H (*J*/Hz)	C(3)·CH$_2$O (*J*/Hz)	C(5′)H (*J*/Hz)	N(2)·CH$_3$	C(9)H$_2$ (*J*/Hz)	OAc	C(6)H$_2$ (*J*/Hz)	C(5′)·CH$_3$ (*J*/Hz)	C(4′) (CH$_3$)$_2$	OH (*J*/Hz)
Sirodesmin A (1)	4.26(s)	5.51(t, 8.5)	5.69, 5.77(q,[b] 12.5)	5.79(q,[b] 6.5)	6.86(s)	6.85, 6.93(q, 16)	7.89(s)	7.42, 8.02(dq,[c] 14, 8.5)	8.70(d,[d] 6.5)	8.97(s) 9.04(s)	6.52(s) 6.55(t, 7.5)
Sirodesmin A [e]	4.32(s)	5.10(t, 9)	4.90, 5.10(q, 12)	5.83(q, 7)	6.85(s)	6.33, 6.51(q, 16)	7.93(s)	7.42, 7.97(dq, 15, 8)	8.76(d, 7)	9.01(s) 9.09(s)	1.07(s[f]) 1.23(s[f])
Sirodesmin A monoacetate (5)	4.25(s)	5.52(t, 8)	5.08, 5.30(q, 12.5)	5.79(q, 6)	6.90(s)	6.90(s)	7.88(s) 7.90(s)	7.44, 8.04(dq, 14, 8)	8.71(d, 6)	8.97(s) 9.05(s)	
Sirodesmin A diacetate (6)	4.36(s)	5.13(t, 9)	5.10, 5.28(q, 12)	5.92(q, 7)	6.90(s)	6.41, 6.61(q, 16)	7.87(s) 7.93(s) 7.97(s)	7.45, 7.97(dq, 14, 9)	8.75(d, 7)	9.00(s) 9.07(s)	
Deacetylsirodesmin A [g] (4)	5.36(d,[h] 9)	5.53(t,[b] 8)	5.67, 5.76(q,[b] 12)	5.70(q,[b] 6.5)	6.85(s)	6.80, 7.34(q, 15)		7.50, 8.04(dq, 14, 8)	8.73(d, 6.5)	8.94(s) 9.07(s)	6.53(s) 6.61(m) 7.13(d, 9)
Dethiosirodesmin A (10)	4.71(s)	6.01(t, 7)	5.71(br[i])	5.88(q, 6)	6.95(s)	7.12, 7.40(q, 15)	7.92(s)	7.65(d, 7)	8.74(d, 6)	9.02(s) 9.09(s)	6.28(s)
Dethiosirodesmin A [e]	4.54(s)	5.63(t, 7)	5.06(s)	6.07(q, 6)	7.01(s)	6.67, 6.79(q, 16)	7.97(s)	7.58, 7.72(dq, 16, 7)	8.81(d, 6)	9.02(s) 9.09(s)	1.27(s[f]) 1.48(s[f])
Sirodesmin B (3)	4.46(s)	5.30(t, 9)	5.74, 6.02(q,[b] 11)	5.84(q,[b] 7)	6.95(s)	6.73, 7.31(q, 15)	7.96(s)	7.50, 8.12(dq, 14, 9)	8.72(d, 7)	9.02(s) 9.07(s)	6.73(s)
Sirodesmin B [e]	4.74(s)	4.59(t, 9.5)	5.17(s)	5.74(q, 7)	6.91(s)	6.33, 6.67(q, 15)	7.87(s)	7.47, 8.01(dq, 14, 9.5)	8.78(d, 7)	8.99(s) 9.05(s)	1.12(s[f]) 1.70(s[f])
Sirodesmin G (7)	4.48(s)	5.70(t, 9)	5.75(d,[j] 8)	6.07(q, 6)	6.87(s)	6.76(s)	7.92(s)	7.25, 8.27(dq,[j] 14, 9)	8.74(d, 6)	8.90(s) 8.98(s)	5.86(s) 6.53(t, 8)
Sirodesmin G [e]	4.37(s)	5.09(dd, 9, 6)	4.88, 5.06(q, 12)	6.14(q, 6)	6.84(s)	6.13, 6.51(q, 17)	7.93(s)	7.33(dd, 15, 9) 8.03(dd, 15, 6)	8.71(d, 6)	8.98(s[k])	
Sirodesmin G diacetate [g] (9)	4.40(s)	5.18(dd, 9, 5)	5.11, 5.25(q, 12)	6.19(q, 7)	6.89(s)	6.25, 6.59(q, 16.5)	7.84(s) 7.96(s[l])	7.47(dd, 15, 9) 8.07(dd, 15, 5)	8.73(d, 7)	9.00(s) 9.04(s)	
Deacetylsirodesmin G (8)	5.49(d,[j] 8)	5.71(dd, 9, 7)	5.73(d,[j] 7)	6.09(t, 6)	6.88(s)	6.75, 7.31(q, 15)		7.33(dd, 15, 9) 8.31(dd, 15, 7)	8.74(d, 6)	8.94(s) 8.99(s)	5.50(s) 6.64(t, 7) 7.18(d, 8)

[a] Unless stated otherwise spectra were measured at 100 MHz for solutions in [^{2}H]chloroform at ambient temperatures. [b] After addition of [^{2}H$_2$]water. [c] Becomes quartet on irradiation at τ 5.51. [d] Becomes singlet on irradiation at τ 5.79. [e] After addition of sufficient trichloroacetyl isocyanate to effect carbamoylation. [f] Signals assigned to NH groups. [g] At 90 MHz. [h] Becomes singlet after addition of [^{2}H$_2$]water or on irradiation at τ 7.13. [i] Becomes singlet after addition of [^{2}H$_2$]water. [j] Becomes quartet on irradiation at τ 5.70. [k] Integral corresponds to 2 × CH$_3$. [l] Integral corresponds to 2 × OAc.

TABLE 2

^{13}C N.m.r. spectra (δ in p.p.m. from Me$_4$Si) for sirodesmins A and G [a]

A	G	
14.6 (q)	14.0 (q)	CH$_3$
17.8 (q)	17.1 (q)	
18.9 (q)	20.2 (q)	
20.2 (q)	20.2 (q)	
27.1 (q)	27.2 (q)	N(2)·CH$_3$
37.5 (t)	33.6 (t)	C(6)
40.8 (t)	43.4 (t)	C(9)
45.8 (s)	47.3 (s)	C(4′)
60.1 (t)	60.5 (t)	C(2)·CH$_2$O
68.2 (d)	67.0 (d)	C(5a)
74.8 (s)	75.1 (s)	C(2), C(9a)
76.4 (s)	77.0 (s)	
79.4 (d)	78.8 (d)	C(5′), C(8)
82.2 (d)	79.9 (d)	
84.4 (s)	82.1 (s)	C(7), C(8a)
89.5 (s)	89.3 (s)	
162.2 (s)	162.6 (s)	C(1), C(4)
165.2 (s)	165.0 (s)	
169.3 (s)	169.7 (s)	C(8)·OCO
216.5 (s)	223.0 (s)	C(3′)

[a] Measured at 22.63 MHz for solutions in [^{2}H$_1$]chloroform at ambient temperatures; multiplicities determined by off-resonance decoupling.

[20] R. Rahman, S. Safe, and A. Taylor, *J.C.S. Perkin I*, 1969, 1665.

[21] R. Nagarajan and R. W. Woody, *J. Amer. Chem. Soc.*, 1973, **95**, 7212.

of sirodesmin G at τ 6.07. With regard to case (*c*), the stereochemistry at positions 5a, 7, 8, 8a, and 5′ can be assigned by the following arguments. First, by analogy with the proposed mechanism for the biosynthesis of the pyrrolidine ring in gliotoxin and related compounds [3,22-25] it is probable that the N(5)–C(5a) bond in the sirodesmins is formed via a *trans*-antiparallel attack of an amine or amide nitrogen atom on an epoxide group. Hence it is unlikely that the 5a-H and 8a-OH are in the *trans*-configuration in sirodesmin G. Secondly, a comparison of the changes in chemical shifts which resulted on conversion of the primary and secondary hydroxy-groups in each of the isomeric metabolites into acetoxy or *N*-trichloroacetylcarbamoyl groups was informative (Table 3). The changes in chemical shifts were calculated from the values given in Table 1 and are quoted to the nearest 0.05 p.p.m., which we regard as the limit to the accuracy of our experiments. In either metabolite derivatisation of the 8a-OH resulted in a downfield shift of $\geqslant$0.4 p.p.m. in the position of the 5a-H resonance together with a comparable downfield shift in that of one of the C-9 protons (presumably that *cis* to the 8a-OH in the parent metabolite). In contrast, the shift in the 8-H resonance in either metabolite was *ca.* 0.1 p.p.m. (though in opposite directions). Thus it appears that the C-8 and C-8a hydroxy-groups are *cis* in both sirodesmins A and G. Dreiding models show that the argument remains valid if the 5a-H and 8a-OH are in the alternative *cis*-configuration in sirodesmin G, *i.e.* on the opposite face of the azabicyclo-octane system to that found in sirodesmin A. If this were the case then the stereochemistry at each of C-5a, C-8, and C-8a in sirodesmin G would be inverted with respect to that found in sirodesmin A. We consider this to be unlikely. The changes in the chemical shifts of corresponding protons in the ^{1}H n.m.r. spectra of sirodesmins A and G induced through derivatisation of the primary and tertiary hydroxy-groups were very similar (Table 3), the chief difference being in the behaviour of the protons at C-6. It would be difficult to account for this difference if sirodesmins A and G were merely epimeric at C-5′. The remaining possibility, that sirodesmins A and G are epimeric at C-7, is compatible with the spectroscopic evidence detailed above since acetylation or carbamoylation of the 8a-OH in sirodesmin G could result in a change in conformation of the cyclopentane ring in order to minimise the steric interaction between the acetate or urethane group at C-8a and the carbonyl group at C-3′. Thirdly, the high resolution i.r. spectrum of a dilute solution of deacetylsirodesmin G [Figure 2(a)] shows bands at 3 380 and 1 735 cm^{-1} [C(3′)=O] characteristic of an intramolecularly hydrogen-bonded system, whereas the spectrum of deacetylsirodesmin A [Figure 2b] lacks the band at 3 380 cm^{-1} and shows a carbonyl band at 1 755 cm^{-1}. The C-3′ resonance occurs at lower field in the ^{13}C n.m.r. spectrum of sirodesmin G than in the spectrum of sirodesmin A (Table 2).

TABLE 3

Changes in chemical shifts ($\Delta\tau$ in p.p.m.) for protons in sirodesmins A and G induced by derivatisation of C(3)·CH$_2$·OH and C(8a)OH

C(3)·CH$_2$OR	C(8)H		C(5a)H		C(3)·CH$_2$O		C(5′)H		N(2)·CH$_3$		C(9)H$_2$		C(6)H$_2$		C(5′)·CH$_3$	
C(8a)OR	A	G	A	G	A	G	A	G	A	G	A	G	A	G	A	G
R = CO·NH·CO·CCl$_3$	0.05	−0.10	−0.40	−0.60	−0.80	−0.85	0.05	−0.05	0.00	−0.05	−0.50	−0.65	0.00	0.10	0.05	−0.05
					−0.70	−0.70					−0.40	−0.25	−0.05	−0.25		
R = Ac	0.10	−0.10	−0.40	−0.50	−0.60	−0.65	0.15	0.10	0.05	0.00	−0.45	−0.50	0.05	0.20	0.05	0.00
					−0.50	−0.50					−0.30	−0.20	−0.05	−0.20		

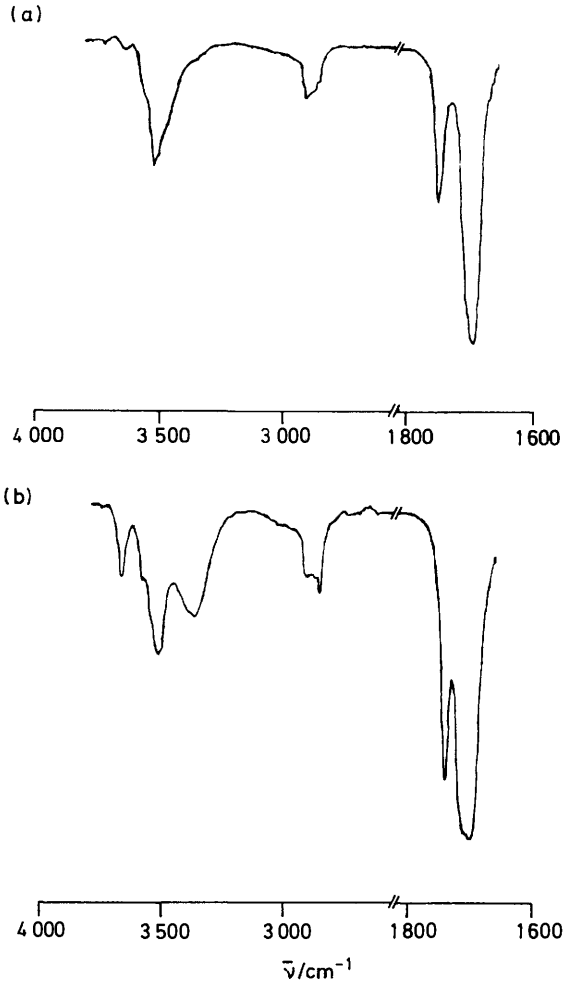


FIGURE 2 I.r. spectra of (a) deacetylsirodesmin A (4) and (b) deacetylsirodesmin G (8) in dichloromethane (path length 1 mm)

[22] M. Neuss, L. D. Boeck, D. R. Brannon, J. C. Cline, D. C. DeLong, M. Gorman, L. L. Huckstep, D. H. Lively, J. Mabe, M. M. Marsh, B. B. Molloy, R. Nagarajan, J. D. Nelson, and W. M. Stark, *Antimicrobial Agents Chemotherapy*, 1968, 213.

[23] J. D. Bu'Lock and A. P. Ryles, *Chem. Comm.*, 1970, 1404.

[24] N. Johns and G. W. Kirby, *Chem. Comm.*, 1971, 163.

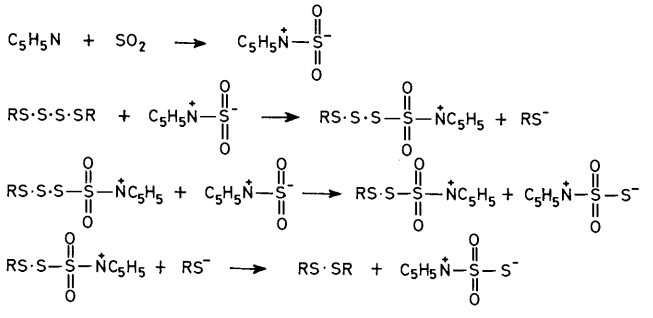
[25] D. R. Brannon, J. A. Mabe, B. B. Molloy, and W. A. Day, *Biochem. Biophys. Res. Comm.*, 1971, **43**, 588.

Again, this indicates that the furanone carbonyl group in sirodesmin G is hydrogen-bonded to an adjacent hydroxy-group.[26] We conclude that sirodesmins A and G are epimeric at C-7 and assign structure (7) to sirodesmin G. The chirality at C-5′ in sirodesmin G remains unknown.

Sirodesmins B and C were converted into sirodesmin A on treatment with sulphur dioxide in pyridine, and this reaction was used to facilitate the isolation of sirodesmin A (and sirodesmin G) from crude extracts of the fermentation liquors. The mechanism by which the desulphurisation reaction proceeds has not been investigated, and there are at least two possibilities. First, as mentioned above, solutions of sirodesmins B or C in pyridine form equilibrium mixtures of sirodesmins A—C and, presumably, sulphur, and it is possible that with the additional presence of sulphur dioxide the composition is shifted in favour of sirodesmin A through removal of sulphur as pyridinium thiosulphate. Secondly, the desulphurisation of polysulphides by cyanide or hydrogen sulphite is known, and a similar mechanism [27] may apply to the pyridine–sulphur dioxide reaction (Scheme 1).

Treatment of sirodesmin A with triphenylphosphine in chloroform gave the monosulphide dethiosirodesmin A (10), identified on the basis of n.m.r. (Table 1) and i.r. data. Of diagnostic value was the shift in the amidic carbonyl stretching frequency from 1 685 in the i.r. spectrum of sirodesmin A to 1 718 cm^{-1} in that of the monosulphide. A similar desulphurisation of dehydrogliotoxin has been reported, and in this case the reaction was thought to occur with inversion at both carbon atoms bonded to sulphur.[28] We are unable to comment as to whether this surprising inversion occurs on formation of dethiosirodesmin A.

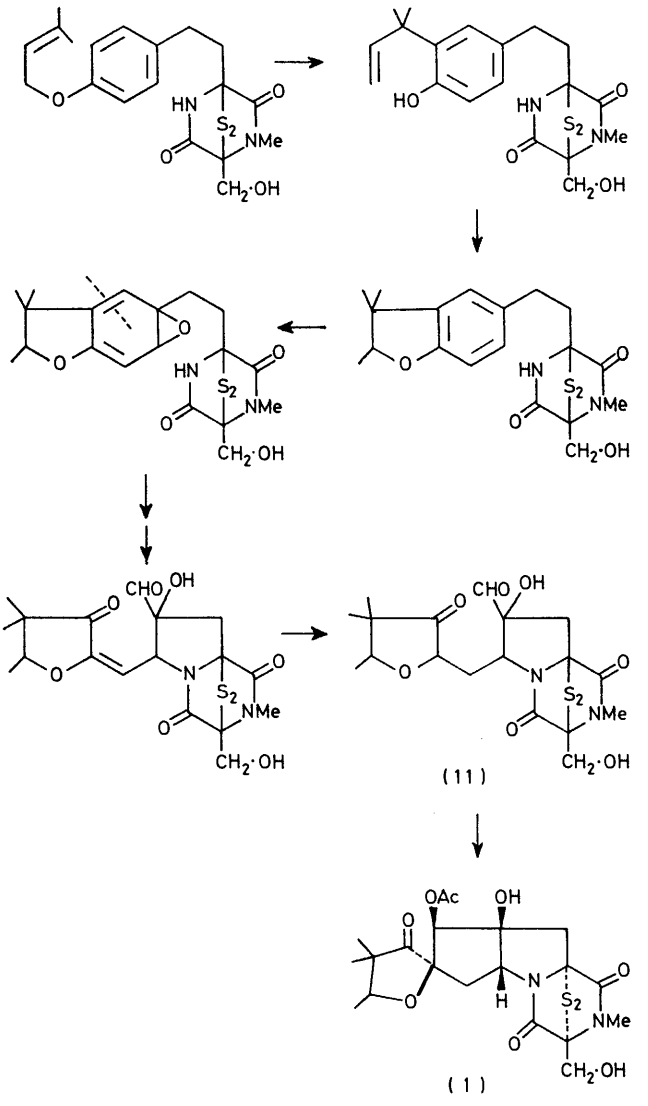
A possible biosynthetic pathway to sirodesmin A is outlined in Scheme 2, and is consistent with postulated pathways to gliotoxin and other epidithiopiperazinediones.[3,22-25] Epimerisation of (11) *via* the derived enol,



SCHEME 1

or the biological equivalent of the enolate anion, could lead to sirodesmin G.

Deacetylsirodesmin A (4), deacetylsirodesmin G (8), sirodesmin A monoacetate (5), sirodesmin A diacetate (6), sirodesmin G diacetate (9), and dethiosirodesmin A (10) have not been detected as natural products.



SCHEME 2 Possible biosynthetic pathway to sirodesmin A

The detailed biological properties of the sirodesmins will be published elsewhere.[29] Sirodesmins A, B, C, and G show exceptionally high activity against small RNA-containing viruses such as the rhinoviruses and the enteroviruses. For example, sirodesmin A, at concentrations of 8 ng ml^{-1}, reduces the growth of picornaviruses in cultures of human diploid lung cells by 50%. In contrast the concentration of sirodesmin A required to produce 50% cytotoxicity in uninfected cultures of the same cells is 1 μg ml^{-1}. However, sirodesmin A has been shown to produce chromosome abnormalities in *in vitro* studies with human lymphocytes and in *in vivo* studies in Chinese hamster bone marrow cells.[30] It

[26] J. B. Stothers, ' Carbon-13 NMR Spectroscopy,' Academic Press, New York, 1972, p. 287.
[27] O. Foss in ' Organic Sulphur Compounds,' ed. N. Kharasch. Pergamon, Oxford, 1961, vol. 1, p. 86.
[28] S. Safe and A. Taylor, *J. Chem. Soc.* (*C*), 1971, 1189.
[29] R. B. Bucknall, in preparation.
[30] M. Richold, K. I. Cinkotai, and J. C. Topham, personal communication.

remains to be established whether this property is peculiar to sirodesmin A or is a more general feature associated with the epipolythiopiperazinedione antibiotics.

Molecular Geometry of Sirodesmin A Diacetate (6).—Relevant bond lengths, interbond angles, and pertinent intra- and inter-molecular non-bonded distances are given in Table 4. Relevant torsion angles are contained

TABLE 4

Some bond lengths (Å), interbond angles (°), and pertinent intra- and inter-molecular non-bonded distances (Å) †

(*a*) Bond lengths

S(10)–S(11)	2.066(2)	C(1)–C(9a)	1.516(6)
S(10)–C(9a)	1.883(4)	C(1)–O(12)	1.212(7)
S(11)–C(3)	1.883(5)	C(3)–C(4)	1.544(7)
N(2)–C(1)	1.362(6)	C(3)–C(14)	1.517(9)
N(2)–C(3)	1.443(7)	C(4)–O(19)	1.212(6)
N(2)–C(13)	1.485(9)	C(5a)–C(8a)	1.551(6)
N(5)–C(4)	1.336(5)	C(8a)–C(9)	1.553(6)
N(5)–C(5a)	1.475(5)	C(9)–C(9a)	1.508(6)
N(5)–C(9a)	1.444(5)		

(*b*) Interbond angles

S(11)–S(10)–C(9a)	97.9(1)	O(19)–C(4)–C(3)	123.5(4)
S(10)–S(11)–C(3)	98.7(2)	N(5)–C(4)–C(3)	111.1(4)
C(1)–N(2)–C(3)	118.2(4)	N(5)–C(5a)–C(6)	112.6(3)
C(1)–N(2)–C(13)	117.5(5)	N(5)–C(5a)–C(8a)	104.2(3)
C(3)–N(2)–C(13)	122.7(5)	C(6)–C(5a)–C(8a)	107.2(3)
C(4)–N(5)–C(5a)	125.4(4)	O(28)–C(8a)–C(8)	111.2(3)
C(4)–N(5)–C(9a)	120.6(4)	O(28)–C(8a)–C(9)	113.6(3)
C(5a)–N(5)–C(9a)	122.7(3)	O(28)–C(8a)–C(5a)	105.9(3)
N(2)–C(1)–C(9a)	112.6(4)	C(5a)–C(8a)–C(8)	103.2(3)
N(2)–C(1)–O(12)	125.0(5)	C(5a)–C(8a)–C(9)	106.0(3)
C(9a)–C(1)–O(12)	122.4(4)	C(8a)–C(9)–C(9a)	104.5(3)
S(11)–C(3)–N(2)	112.2(4)	S(10)–C(9a)–N(5)	111.1(3)
S(11)–C(3)–C(4)	102.6(3)	S(10)–C(9a)–C(1)	102.4(3)
S(11)–C(3)–C(14)	106.6(4)	S(10)–C(9a)–C(9)	111.0(3)
N(2)–C(3)–C(4)	110.5(4)	N(5)–C(9a)–C(1)	111.0(3)
N(2)–C(3)–C(14)	115.9(5)	N(5)–C(9a)–C(9)	106.0(3)
C(4)–C(3)–C(14)	108.2(5)	C(1)–C(9a)–C(9)	115.4(4)
O(19)–C(4)–N(5)	125.4(4)		

(*c*) Pertinent intramolecular non-bonded distances <3.30 Å

S(10) · · · N(2)	3.15	S(11) · · · C(1)	3.23
S(10) · · · C(4)	3.27	C(3) · · · C(9a)	2.64
S(11) · · · N(5)	3.10		

(*d*) Intermolecular distances <3.60 Å

C(31) · · · S(11)^{I}	3.58	O(1′) · · · C(13)^{V}	3.58
C(21) · · · O(26)^{II}	3.54	O(19) · · · C(9)^{V}	3.59
C(21) · · · O(30)^{II}	3.29	O(19) · · · C(29)^{V}	3.44
C(5′) · · · O(20)^{III}	3.44	O(28) · · · C(13)^{V}	3.50
C(23) · · · O(20)^{III}	3.36	C(5a) · · · O(12)^{V}	3.26
C(18) · · · O(12)^{IV}	3.29	C(27) · · · O(17)^{VI}	3.14

Roman numerals as superscripts refer to the following equivalent positions which should be applied to the co-ordinates of the second atom:

I	x	y	$1 + z$
II	$1 + x$	y	z
III	$1\frac{1}{2} - x$	$1 - y$	$\frac{1}{2} + z$
IV	$\frac{1}{2} + x$	$\frac{1}{2} - y$	$-z$
V	$\frac{1}{2} + x$	$\frac{1}{2} - y$	$1 - z$
VI	$1 - x$	$\frac{1}{2} + y$	$\frac{1}{2} - z$

† A complete set of dimensions has been deposited with the structure-factor tables.

in Table 5. The atomic numbering is defined in formula (12), hydrogen atoms being numbered as the carbon atoms to which they are bonded.

The constraints of the disulphide bridge force the piperazinedione ring of sirodesmin A diacetate (6) to adopt a boat conformation, although an examination of the relevant endocyclic torsion angles reveals that this conformation is both flattened and slightly twisted.

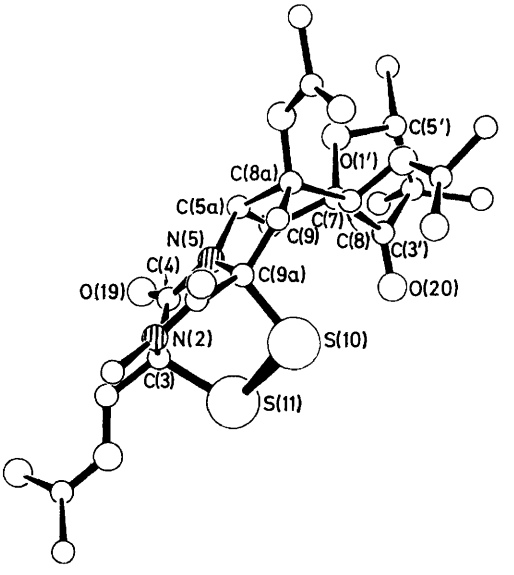
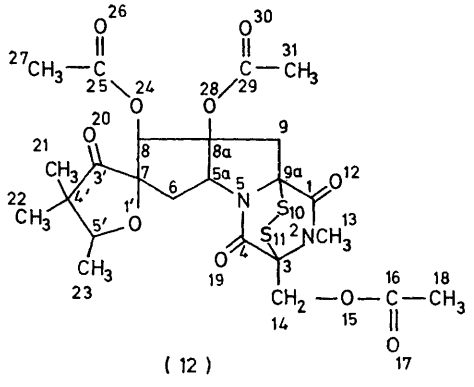


FIGURE 3 A projected view of one molecule of sirodesmin A diacetate (6), illustrating the solid state conformation

TABLE 5

Selected torsion angles (°)

Piperazinedione ring	
C(3)–S(11)–S(10)–C(9a)	+11.4(2)
C(3)–N(2)–C(1)–C(9a)	−5.8(6)
N(2)–C(1)–C(9a)–N(5)	−41.6(5)
C(1)–C(9a)–N(5)–C(4)	+46.5(5)
C(9a)–N(5)–C(4)–C(3)	−2.2(6)
N(5)–C(4)–C(3)–N(2)	−45.4(5)
C(4)–C(3)–N(2)–C(1)	+50.0(6)
Pyrrolidine ring	
C(5a)–N(5)–C(9a)–C(9)	−20.2(4)
N(5)–C(9a)–C(9)–C(8a)	+25.8(4)
C(9a)–C(9)–C(8a)–C(5a)	−22.6(4)
C(9)–C(8a)–C(5a)–N(5)	+11.0(4)
C(8a)–C(5a)–N(5)–C(9a)	+5.4(4)



(12)

Despite the adoption of a distorted boat conformation, the planar geometries of C(1) and C(4) are maintained, while N(2) and N(5) deviate only slightly from planarity. The disulphide bridge adopts a slightly skewed orientation with respect to the piperazinedione ring, being compressed, while the angles N(5)C(9a)S(10) and N(2)C(3)S(11) have magnitudes of 111.1(3) and 112.2(4)°,

respectively. The C(3)S(11)S(10)C(9a) torsion angle is 11.4(2)°, and the values of the C(9a)S(10)S(11) and C(3)S(11)S(10) valence angles are respectively 97.9(1) and 98.7(2)°. The C(3)–S(11) and C(9a)–S(10) bond lengths are identically 1.883(5) Å, and the S(10)–S(11) bond has a length of 2.066(2) Å. The above geometry of the piperazinedione ring closely parallels those geometries observed for the same moiety in several other natural products, notably acetylaranotin [5] (LL-588α), gliotoxin,[31] and sporidesmin.[32] The skewed orientation of the disulphide bridge with respect to the piperazinedione ring is also observed in the other three examples, and in each case is characterised by compression of the two (O)CCS valence angles, and by similar values for the CSSC torsion angle.

An examination of the relevant endocyclic torsion angles reveals that the pyrrolidine ring of the present molecule adopts a flattened and twisted envelope conformation, in which C(9) may nevertheless be identified as the out-of-plane atom. A similarly twisted, but slightly less flattened envelope conformation is observed for the *cis*-fused cyclopentane ring, C(7) being the out-of-plane atom. It seems probable that the conformations of these two rings are largely dominated not only by their *cis*-fusion to each other, but also by the constraints of the other ring systems and substituents to which they are individually bonded. Thus, we note that the ester groups attached to C(8) and C(8a) are in close proximity, while O(1′) of the spiro-fused tetrahydrofuran-3-one system closely approaches the ester group bonded to C(8). The latter ring also adopts a distorted envelope conformation in which C(5′) may be recognised as the out-of-plane atom.

Other features of the molecular geometry are unremarkable: the acetate groups are acceptably planar, and bond lengths and interbond angles conform to values which would be expected for similar bonding situations. There are no abnormally short intermolecular distances, and it is assumed that packing is largely dominated by van der Waals forces.

EXPERIMENTAL

I.r. spectra were determined with a Perkin-Elmer 457 double-beam grating spectrometer. Mass spectrometric data were determined with A.E.I. MS9 and Hitachi RMU-6E spectrometers. N.m.r. spectra were determined with Varian HA100D and Bruker HX90E spectrometers. C.d. spectra were determined with a Carey 60 spectrometer. M.p.s were recorded with a Kofler hot-stage apparatus. Petroleum refers to the fraction of b.p. 60—80 °C. Silica gel used for column chromatography was Hopkin and Williams MFC and contained 12% water. T.l.c. was performed on Merck silica gel GF 254; solvent systems were toluene–ethyl acetate (1 : 2) (I), chloroform–methanol–formic acid (95 : 4 : 1) (II), and chloroform–methanol (95 : 5) (III). For preparative layer chromatography (p.l.c.) the layers were of dimensions 40 × 20 × 0.1 cm. The R_F values of the sirodesmins and their derivatives in systems I and II are given in Table 6. Sirodesmins A, B, C, and G were amorphous, and the identities of samples of the metabolites obtained from interconversion studies were confirmed on the basis of n.m.r. data.

Isolation of the Metabolites.—(a) *Sirodesmins* A (1), B (3), C (2), *and* G(7). *Sirodesmium diversum* (CMI 102519, no. 2 191 in our collection) was grown as surface culture for 35 days in glass vessels each containing 1 l of the following medium (concentrations in g l^{-1}) adjusted to pH 6.5: cerelose (50), sodium nitrate (2), potassium dihydrogen phosphate (1), magnesium sulphate heptahydrate (0.5), potassium chloride (0.5), iron(II) sulphate (0.01), oxoid yeast extract (1), and 0.1% (v/v) minor element concentrate.[33] The culture filtrate (120 l) was extracted with chloroform to afford a gum which was fractionated in a simplified counter-current procedure utilising two separating funnels and the two-phase solvent system methanol–toluene–chloroform–water (9 : 9 : 4 : 4). The upper, stationary phases (2 × 100 ml) were discarded and the lower, mobile phases (7 × 200 ml) combined to yield a gum which was further fractionated by a similar procedure in the solvent system toluene–petroleum–methanol–water (3 : 2 : 4 : 1). Chromatography of the material (10 g) from the pooled lower phases on silica gel (850 g) afforded fractions A [ethyl acetate–toluene (1 : 4); 4.5 l], B [ethyl acetate–toluene (3 : 7); 2.0 l], C [ethyl acetate–toluene (3 : 7); 1.7 l], D [ethyl acetate–toluene (2 : 3); 4.6 l], E [ethyl acetate–toluene (1 : 1); 1.4 l], F [ethyl acetate–toluene (1 : 1); 5.0 l], G [ethyl acetate–toluene (1 : 1); 1.4 l]; H [ethyl acetate (1.8 l)]. and I [ethyl acetate (5.6 l)]. Repeated p.l.c. of fraction C (0.26 g), initially in system I, then in system II, afforded *sirodesmin* G which was precipitated from ether by petroleum as an amorphous solid (38 mg) [Found: C, 49.7; H, 5.4; N, 5.7; S, 12.6%; $(M - S_2)^+$, 422. $C_{20}H_{26}N_2O_8S_2$ requires C, 49.4; H, 5.4; N, 5.8; S, 13.2%; $(M - S_2)$, 422]. P.l.c. of fraction E (0.5 g) in system II afforded *sirodesmin* A which was precipitated from ether by petroleum as an amorphous solid (175 mg) [Found: C, 49.7; H, 5.4; N, 5.8; S, 12.8%; $(M - S_2)^+$, 422.173 1. $C_{20}H_{26}N_2O_8S_2$ requires C, 49.4; H, 5.4; N, 5.8; S, 13.2%; $(M - S_2)$, 422.168 9]. P.l.c. of fraction F (2 g) in system III, then in system II, afforded *sirodesmin* A (90 mg) and *sirodesmin* C. The latter was precipitated from ether by petroleum as an amorphous solid (160 mg) [Found: C, 46.3; H, 5.1; N, 5.5; S, 18.0%; $(M - S_3)^+$, 422. $C_{20}H_{26}N_2O_8S_3$ requires C, 46.3; H, 5.0;

TABLE 6

R_F Values for the sirodesmins and derivatives [a]

	System I [b]	System II [b]
Sirodesmin A (1)	0.23	0.35
Sirodesmin B (3)	0.07	0.24
Sirodesmin C (2)	0.17	0.29
Sirodesmin G (7)	0.38	0.39
Deacetylsirodesmin A (4)	0.23	0.24
Deacetylsirodesmin G (8)	0.33	0.27
Sirodesmin A monoacetate (5)	0.31	0.45
Sirodesmin A diacetate (6)	0.39	0.51
Sirodesmin G diacetate (9)	0.52	0.55
Dethiosirodesmin A (10)	0.24	0.29

[a] On Merck GF 254 silica gel. [b] See text.

[31] J. Fridrichsons and A. M. Mathieson, *Acta Cryst.*, 1967, **23**, 439.

[32] J. Fridrichsons and A. M. Mathieson, *Acta Cryst.*, 1965, **18**, 1043.

[33] P. W. Brian, P. J. Curtis, and H. G. Hemming, *Trans. Brit. Mycol. Soc.*, 1946, **29**. 173.

N, 5.4; S, 18.5%; ($M - S_3$), 422]. Fraction I (2.3 g) was adsorbed on silica gel (220 g). Chloroform–methanol (99.5 : 0.5) eluted *sirodesmin* B, which was precipitated from ether by petroleum as an amorphous solid (1.8 g) [Found: C, 43.4; H, 4.9; N, 4.9; S, 22.8%; $(M - S_4)^+$, 422. $C_{20}H_{26}N_2O_8S_4$ requires C, 43.6; H, 4.8; N, 5.1; S, 23.3%; ($M - S_4$), 422].

(b) *Sirodesmins* A (1) *and* G (7). Extraction of the culture filtrate (300 l) with chloroform afforded a gum (54.3 g) which was dissolved in pyridine (100 ml) and treated for 2.5 h at room temperature with a solution prepared by passing sulphur dioxide through pyridine (320 ml) at 0 °C until the volume had increased to 400 ml. Toluene (2 l) and ice (500 g) were added and the mixture was acidified with 3N-sulphuric acid (900 ml). The organic layer was separated and the aqueous layer was extracted with toluene (2 × 2 l). The combined organic extracts were washed with water (600 ml), dried (Na_2SO_4), then concentrated to yield a gum which was separated by chromatography on silica gel (1 050 g). Toluene–ethyl acetate (3 : 1) (5 l) eluted fraction A; toluene–ethyl acetate (7 : 3), (5 l) eluted fraction B followed by fraction C; toluene–ethyl acetate (65 : 35), (5 l) eluted fraction D followed by fraction E; and toluene–ethyl acetate (3 : 2), (6.5 l) eluted fraction F. Fractions A and B were combined (3.67 g) then separated further by chromatography on silica gel (250 g). Chloroform–ethanol (99.5 : 0.5) (2.1 l) followed by chloroform–ethanol (99 : 1) (0.5 l) eluted fraction G, which was separated by p.l.c. in system II to afford sirodesmin G (652 mg.). Fractions E and F were pooled (9.4 g), then separated by chromatography on silica gel (1 kg). Chloroform–ethanol (49 : 1) (2 l) eluted sirodesmin A (4.87 g).

Similar fermentations, which were worked up by this method but for sirodesmin A alone, afforded the metabolite in yields of *ca.* 40 mg l^{-1} of culture filtrate.

Desulphurisation of Sirodesmin C (2) *with Sulphur Dioxide*.—Sulphur dioxide was passed through pyridine (1.0 ml) at 0 °C until the volume had increased to 1.7 ml. Sirodesmin C (96 mg) was added and the mixture was left at room temperature for 1.6 h. Chloroform (10 ml) was added and the mixture was washed with N-sulphuric acid (10 ml) and water (10 ml), then dried (Na_2SO_4) and evaporated. The residual gum (70 mg) was separated by p.l.c. in system II to afford sirodesmin A (45 mg).

Desulphurisation of Sirodesmin B with Sulphur Dioxide.—Sulphur dioxide was passed through pyridine (18 ml) at 0 °C until the volume had increased to 24 ml. Sirodesmin B (116 mg) was added and the mixture was kept at room temperature for 3 h. Chloroform (100 ml) was added and the mixture was washed with N-sulphuric acid (100 ml), 0.6N-sulphuric acid (50 ml), and water (50 ml), then dried (Na_2SO_4) and evaporated. The residual gum was separated by p.l.c. in system II to afford sirodesmin A (82 mg.).

Conversion of Sirodesmin A (1) *into Sirodesmins* B (3) *and* C (2).—A stirred mixture of sirodesmin A (150 mg), sulphur (40.5 mg), and pyridine (26 ml) was kept at room temperature for 25 h. Evaporation and separation of the residue by repeated p.l.c. in system II afforded sirodesmins B (55 mg) and C (37 mg).

Deacetylsirodesmin A (4).—A solution of sirodesmin A (83 mg) in methanol (100 ml) and aqueous 36.5% hydrochloric acid (1 ml) was kept for 4 days at room temperature. The solvents were evaporated off and the residue was separated by p.l.c. in system II. Crystallisation of the product from ethyl acetate–petroleum gave *deacetylsirodesmin A* as needles, m.p. 193—196° (Found: C, 48.4; H, 5.6; N, 6.0; S, 14.3. $C_{18}H_{24}N_2O_7S_2$ requires C, 48.8; H, 5.5; N, 6.3; S, 14.4%).

Deacetylsirodesmin G (8).—A solution of sirodesmin G (100 mg) in methanol (100 ml) and aqueous 36.5% hydrochloric acid (1 ml) was kept for 5 days at room temperature. The solvents were evaporated off, water (25 ml) was added, and the mixture was extracted with chloroform (3 × 25 ml). Separation of the chloroform-soluble material by p.l.c. in system III and crystallisation of the product from acetone gave *deacetylsirodesmin* G as needles (40 mg), m.p. 195—196.5° (Found: C, 48.4; H, 5.5; N, 6.5; S, 14.6. $C_{18}H_{24}N_2O_7S_2$ requires C, 48.8; H, 5.5; N, 6.3; S, 14.4%).

Sirodesmin A *Monoacetate* (5).—A solution of sirodesmin A (92 mg) and acetic anhydride (3 ml) in pyridine (6 ml) was left for 1 h at room temperature, then poured on to ice. The mixture was acidified with 2N-hydrochloric acid (35 ml) and extracted with chloroform (2 × 20 ml). The extracts were washed with aqueous 5% sodium hydrogen carbonate (5 ml) and water (2 × 20 ml), then dried (Na_2SO_4) and evaporated. The residue was separated by p.l.c. in system I. Precipitation of the product from ether with petroleum afforded *sirodesmin* A *monoacetate* (66 mg) as an amorphous solid [Found: C, 49.9; H, 5.3; N, 5.3; S, 11.7%; $(M - S)^+$, 496. $C_{22}H_{28}N_2O_9S_2$ requires C, 50.0; H, 5.3; N, 5.2; S, 12.1%; ($M - S$), 496].

Sirodesmin A *Diacetate* (6).—A solution of sirodesmin A (250 mg) and acetic anhydride (6.25 ml) in pyridine (9.5 ml) was left for 1 week at room temperature, then cooled to 0 °C. Water (1.25 ml) was added and the mixture was allowed to warm to room temperature over 0.5 h, then acidified to pH 2 with 2N-sulphuric acid and extracted with ethyl acetate (4 × 50 ml). The extracts were washed with water (2 × 25 ml), followed by 2% sodium hydrogen carbonate (2 × 25 ml), then dried (Na_2SO_4) and evaporated. P.l.c. of the residue in system II afforded *sirodesmin* A *diacetate* (177 mg), which was crystallised twice from ethyl acetate–petroleum to give needles (127 mg), m.p. 186—189° [Found: C, 50.9; H, 5.4; N, 4.6; S, 11.4%; $(M - S_2)^+$, 506.187 1. $C_{24}H_{30}N_2O_{10}S_2$ requires C, 50.5; H, 5.3; N, 4.9; S, 11.2%; ($M - S_2$), 506.190 1].

Sirodesmin G *Diacetate* (9).—A solution of sirodesmin G (44 mg) and acetic anhydride (1 ml) in pyridine (2 ml) was left for 9 days at room temperature, then cooled to 0 °C. Water (0.4 ml) was added and the mixture allowed to warm to room temperature over 0.5 h, then acidified with 3N-sulphuric acid (60 ml) and extracted with ethyl acetate (3 × 25 ml). The extracts were washed with water (50 ml), aqueous 2% sodium hydrogen carbonate (2 × 50 ml), and water (2 × 50 ml), then dried (Na_2SO_4) and evaporated. Recrystallisation of the product from ethyl acetate–petroleum afforded *sirodesmin G diacetate* as needles, m.p. 159.5—161° (Found: C, 50.7; H, 5.3; N, 4.7; S, 11.0. $C_{24}H_{30}N_2O_{10}S_2$ requires C, 50.5; H, 5.3; N, 4.9; S, 11.2%).

Dethiosirodesmin A (10).—A solution of sirodesmin A (150 mg) and triphenylphosphine (79 mg) in chloroform (6 ml) was kept for 4 h at room temperature. Separation of the product by p.l.c. in system II afforded *dethiosirodesmin* A, which was precipitated from ethyl acetate by petroleum as an amorphous solid (51.5 mg) (Found: C, 52.7; H, 5.7; N, 5.8; S, 7.1%; M^+, 454.137 6. $C_{20}H_{26}N_2O_8S$ requires C, 52.9; H, 5.8; N, 6.2; S, 7.0%; *M*, 454.105 6), $\nu_{max.}$ ($CHCl_3$) 1 763 and 1 718 cm^{-1}.

X-Ray Structure Determination of Sirodesmin A *Diacetate*

(6).—*Crystal data.* $C_{24}H_{30}N_2O_{10}S_2$. M = 570.7. Orthorhombic, a = 9.756(1), b = 25.114(2), C = 11.372(1) Å, U = 2 786.3 Å^3, D_m = 1.35 (by flotation), Z = 4, D_c = 1.36 g cm^{-3}, F (000) = 1 200. Space group $P2_12_12_1$ from systematic absences. Mo-K_α X-radiation, λ = 0.710 7 Å, μ(Mo-K_α) = 2.5 cm^{-1}.

TABLE 7

Fractional co-ordinates and temperature factors (Å^2)

(*a*) Fractional co-ordinates (×10^4 for non-hydrogen atoms; ×10^3 for hydrogen atoms)

Atom	*x*	*y*	*z*
S(10)	3 851(1)	3 654(1)	2 288(1)
S(11)	4 801(2)	3 119(1)	1 174(1)
O(1′)	7 447(3)	3 987(1)	6 203(2)
O(12)	1 335(4)	2 840(2)	3 414(3)
O(15)	4 450(5)	2 057(2)	135(4)
O(17)	5 189(17)	1 295(4)	−173(7)
O(19)	6 484(4)	2 338(1)	3 250(3)
O(20)	7 586(4)	4 493(2)	3 359(3)
O(24)	5 019(3)	4 551(1)	5 773(3)
O(26)	3 451(4)	4 848(2)	4 497(4)
O(28)	4 502(3)	3 527(1)	6 572(2)
O(30)	2 611(4)	4 032(2)	6 733(3)
N(2)	3 056(5)	2 441(2)	2 378(4)
N(5)	4 863(4)	2 920(1)	3 865(3)
C(1)	2 529(6)	2 810(2)	3 129(4)
C(3)	4 473(6)	2 491(2)	2 031(4)
C(3′)	7 727(5)	4 399(2)	4 381(4)
C(4)	5 397(6)	2 564(2)	3 122(4)
C(4′)	8 730(5)	4 654(2)	5 222(5)
C(5a)	5 585(5)	3 160(2)	4 873(3)
C(5′)	8 117(5)	4 498(2)	6 410(5)
C(6)	6 928(5)	3 439(2)	4 512(4)
C(7)	6 890(4)	3 987(2)	5 060(4)
C(8)	5 339(4)	4 114(2)	5 010(4)
C(8a)	4 592(4)	3 598(2)	5 318(3)
C(9)	3 214(4)	3 503(2)	4 667(4)
C(9a)	3 606(4)	3 195(2)	3 579(3)
C(13)	2 073(9)	2 089(3)	1 753(7)
C(14)	5 047(9)	2 036(3)	1 303(6)
C(16)	4 604(9)	1 688(3)	−525(6)
C(18)	3 948(11)	1 675(3)	−1 671(7)
C(21)	10 090(6)	4 357(5)	4 947(7)
C(22)	8 829(14)	5 249(4)	5 029(10)
C(23)	9 128(6)	4 429(3)	7 419(6)
C(25)	4 004(5)	4 884(2)	5 438(6)
C(27)	3 623(10)	5 265(4)	6 392(10)
C(29)	3 493(5)	3 773(2)	7 176(4)
C(31)	3 639(9)	3 678(4)	8 455(5)
H(5a)	553(6)	280(2)	539(5)
H(5′)	743(7)	484(2)	655(5)
H(61)	670(6)	367(2)	376(5)
H(62)	756(7)	326(2)	475(5)
H(8)	510(6)	420(2)	417(4)
H(91)	281(6)	389(2)	437(4)
H(92)	261(6)	323(2)	521(4)
H(131)	253(9)	179(3)	163(7)
H(132)	202(8)	219(3)	105(8)
H(133)	124(8)	226(3)	211(7)
H(141)	478(9)	167(3)	182(6)
H(142)	625(9)	216(3)	115(7)
H(181)	342(8)	129(3)	−183(6)
H(182)	383(12)	184(3)	−180(9)
H(211)	987(9)	388(3)	534(7)
H(212)	1 046(8)	480(3)	546(7)
H(213)	1 034(9)	450(3)	412(8)
H(221)	797(10)	525(4)	513(9)
H(222)	906(12)	527(4)	432(8)
H(223)	961(9)	533(3)	584(7)
H(231)	971(7)	483(3)	769(6)
H(232)	952(7)	405(3)	752(6)
H(233)	869(8)	439(3)	811(6)
H(271)	412(15)	533(5)	657(13)
H(272)	308(8)	507(3)	711(7)
H(273)	442(11)	549(4)	680(11)
H(311)	446(8)	369(3)	872(6)
H(312)	273(8)	391(3)	874(6)
H(313)	367(11)	345(3)	859(8)

TABLE 7 (*Continued*)

(*b*) Anisotropic temperature factors (Å^2 × 10^4) *

Atom	U_{11}	U_{22}	U_{33}	$2U_{12}$	$2U_{31}$	$2U_{23}$
S(10)	679	653	554	63	−30	166
S(11)	885	944	523	137	37	66
O(1′)	490	670	534	−17	−46	31
O(12)	623	935	725	−197	−79	124
O(15)	1 268	1 151	752	254	−388	−337
O(17)	5 346	2 526	1 486	2 275	−1 489	−1 163
O(19)	845	751	656	294	−215	−139
O(20)	783	1 232	688	−97	83	211
O(24)	494	585	742	72	−15	−117
O(26)	735	871	1 176	215	−91	222
O(28)	504	688	477	27	−19	32
O(30)	557	1 026	655	192	15	−25
N(2)	852	634	632	−119	−212	−1
N(5)	584	551	489	721	−141	32
C(1)	711	678	525	−89	−152	119
C(3)	817	669	609	104	−190	−34
C(3′)	419	857	604	46	36	121
C(4)	793	531	507	116	−142	−4
C(4′)	547	933	928	−181	−144	206
C(5a)	564	592	445	61	−86	−31
C(5′)	572	727	767	−7	−134	−88
C(6)	462	731	745	85	2	−121
C(7)	423	627	568	59	15	2
C(8)	447	617	486	59	−4	−31
C(8a)	459	601	446	32	−29	43
C(9)	432	694	531	86	−59	43
C(9a)	522	549	495	−2	−88	90
C(13)	1 039	922	864	−318	−211	−161
C(14)	1 233	1 024	727	255	−406	−294
C(16)	1 236	1 182	746	359	−120	−241
C(18)	1 061	1 449	625	−187	−78	−256
C(21)	412	2 306	1 073	−8	18	397
C(22)	1 572	1 098	1 592	−645	−525	404
C(23)	707	1 124	750	39	−218	−140
C(25)	539	650	1 046	103	40	61
C(27)	969	841	1 885	251	114	−339
C(29)	524	834	561	−93	67	−15
C(31)	803	1 497	567	−6	37	64

(*c*) Average e.s.d.s (Å^2 × 10^4) for anisotropic temperature factors

Atom	U_{11}	U_{22}	U_{33}	$2U_{12}$	$2U_{31}$	$2U_{23}$
S	8	7	6	7	6	5
O	48	29	25	33	29	25
N	23	18	20	18	29	17
C	36	37	36	35	31	32

* Anisotropic thermal parameters are in the form: $\exp[-2\pi^2(U_{11}h^2a^{*2} + U_{22}k^2b^2 + U_{33}l^2c^{*2} + 2U_{23}klb^*c^* + 2U_{31}lhc^*a^* + 2U_{12}hka^*b^*)]$.

Crystallographic measurements, structure determination, and refinement. Unit-cell parameters were initially determined photographically and were subsequently refined by least-squares calculations prior to the diffractometer data collection. The space group is uniquely determined from systematic absences in the X-ray spectra.

Intensity measurements were made on a Hilger and Watts Y290 diffractometer; a small crystal mounted about the a axis was exposed to Zr-filtered Mo-K_α radiation. In all, 2 360 independent reflections ($I > 3\sigma_I$; $\sigma_I = \sqrt{I + B_1 + B_2}$) were recorded by using the θ, ω-scan technique in the range 2θ 0—50°. The intensities were corrected for Lorentz and polarisation factors, but absorption effects were considered small and were ignored.

The structure was solved by the symbolic addition method, by using the programs SINGEN and TANGEN incorporated into the X-RAY suite of programs.[34] An

[34] 'The X-Ray System of Crystallographic Programs,' eds. J. M. Stewart, G. J. Kruger, H. L. Ammon, C. Dickinson, and S. R. Hall, Technical Report TR-192, Computer Science Center, University of Maryland, June 1972.

initial E-map based on 320 reflections with $|E| \geqslant 1.50$ revealed most of the non-hydrogen atoms, the remainder of the structure being determined by conventional structure-factor and electron-density calculations.

Atomic parameters were refined by full-matrix least-squares calculations which converged after 11 cycles, when R was 0.051. All but one of the hydrogen atoms were located from a difference synthesis, and contributions from these atoms were subsequently included in the least-squares calculations. On convergence of the least-squares calculations, calculation of a difference synthesis revealed no errors in the structure, and the refinement was considered complete.

Final fractional co-ordinates and thermal parameters are presented in Table 7. The e.s.d.s in Tables 4, 5, and 7 are derived from the inverse of the least-squares normal-equation matrix, and should be regarded as minimum values. Figure 3 shows a view of one molecule, illustrating the crystal-state conformation. Observed and calculated structure factors are available as Supplementary Publication No. SUP 21905 (28 pp., 1 microfiche).*

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* For details of Supplementary Publications see Notice to Authors No. 7, *J.C.S. Perkin I*, 1975, Index issue.