

## Studies on Fungal Products. Part 8.<sup>1</sup> Isolation and Structure of Emestrin, a Novel Antifungal Macrocyclic Epidithiodioxopiperazine from *Emericella striata*. X-Ray Molecular Structure of Emestrin

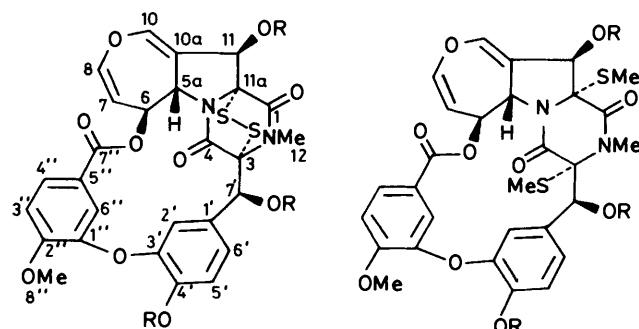
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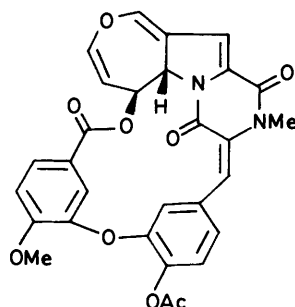
Together with ergosterol, sterigmatocystin, and emericellin, a new compound designated emestrin (**1**),  $C_{27}H_{22}H_2O_{10}S_2$ , was isolated from the mycelial extract of *Emericella striata*. Violaceic acid (**6**) was also isolated from the culture filtrate. The structure of emestrin (**1**), including the absolute configuration, was determined on the basis of a spectroscopic investigation of some derivatives and X-ray crystallography of emestrin methanol solvate. Emestrin is a new macrocyclic epidithiodioxopiperazine, derived from two molecules of phenylalanine and one molecule of benzoic acid, and has strong antifungal activity.

*Emericella striata* (Rai, Tewari & Mukerji) Malloch & Cain, strain 80-NE-22,<sup>2</sup> is a thermotolerant fungus isolated from cumin (the seeds of *Cuminum cyminum* L.) collected in Nepal. A new compound (**1**),<sup>3</sup> along with ergosterol, sterigmatocystin, and emericellin, was isolated from the mycelial acetone extract



(1) R = H  
(2) R = Ac

(3) R = H  
(4) R = Ac



(5)

of this fungus. From the acidic extract of the culture filtrate, we also isolated violaceic acid (**6**), which Yamazaki and Maebayashi isolated from *Emericella violacea* a couple of years ago.<sup>4</sup> The structural elucidation of the compound (**1**), which was designated emestrin, is reported in this paper.

### Results and Discussion

Emestrin (**1**), m.p. 233–236 °C,  $[\alpha]_D + 184^\circ$ , gave a molecular ion at  $m/z$  598 by field desorption (FD) mass spectrometry, and elemental analysis confirmed the empirical formula as  $C_{27}H_{22}N_2O_{10}S_2$ . A positive silver nitrate test (dark brown)<sup>5</sup> and the ion at  $m/z$  534  $[(M - S_2)^+]$  in the FD mass spectrum suggested the presence of a dithio bond in emestrin. The absorptions at 1710, and at 1680 and 1660  $cm^{-1}$  in the i.r. spectrum of emestrin suggested the presence of an ester and amides, respectively. <sup>13</sup>C N.m.r. signals at  $\delta_C$  164.43, 164.28, and 160.25 p.p.m. for emestrin were assigned to one ester and two amide carbons in view of the presence of the two nitrogen atoms in the molecule.

On acetylation, emestrin afforded a triacetate (**2**), m.p. 215–217 °C,  $[\alpha]_D - 27.4^\circ$ ,  $C_{33}H_{28}N_2O_{13}S_2$ , which showed <sup>1</sup>H n.m.r. signals at  $\delta_H$  2.02, 2.10, and 2.29 assigned to the methyl protons of two aliphatic acetoxy groups and those of an aromatic acetoxy group. The <sup>1</sup>H n.m.r. signals of the protons attached to the carbons bearing hydroxy groups at  $\delta_H$  4.967 (d,  $J$  4.1 Hz) and 5.466 (d,  $J$  7.1 Hz) in emestrin shifted downfield to  $\delta_H$  6.44 (s) and 6.63 (s) after acetylation. These results suggested the presence of two alcohols and one phenol in emestrin.

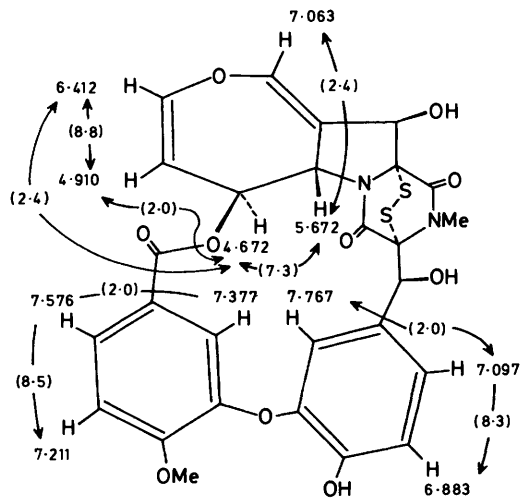
Reductive methylation<sup>6</sup> of emestrin and its diacetate (**2**) gave didithiobis(methylthio)emestrin (**3**), m.p. 184–186 °C,  $C_{29}H_{28}N_2O_{10}S_2$ , and triacetyl didithiobis(methylthio)emestrin (**4**), m.p. 225–227 °C,  $C_{35}H_{34}N_2O_{13}S_2$ , respectively. Both of these compounds have two methylthio groups in the molecule, because <sup>1</sup>H n.m.r. signals at  $\delta_H$  1.907 and 2.275 in (**3**) and at  $\delta_H$  1.50 and 1.98 and (**4**), and <sup>13</sup>C n.m.r. signals at  $\delta_C$  14.5 and 14.8 p.p.m. in (**4**) are observed. The c.d. curve of emestrin was closely similar to those of epidithiodioxopiperazines,<sup>7</sup> as described later. <sup>1</sup>H n.m.r. signals at  $\delta_H$  3.255 in emestrin were assigned as a methyl group attached to the nitrogen atom of the amide. Thus the structure of emestrin was suggested to contain an epidithiodioxopiperazine moiety with two nitrogen atoms, one methylated and the other trisubstituted.

Compound (**5**), m.p. 282–285 °C,  $C_{29}H_{22}N_2O_9$ , was derived from the triacetate (**2**) either by treatment with activated Raney nickel<sup>8</sup> or by basic hydrolysis. It is likely that deacetylation and desulphurization occurred simultaneously giving rise to compound (**5**). The <sup>1</sup>H n.m.r. signals at  $\delta_H$  4.967 and 5.466 in emestrin, assigned to protons attached to carbons bearing a hydroxy group, were apparently shifted to  $\delta_H$  6.67 (s) and 6.52 (s) in compound (**5**). Only one phenolic acetoxy signal, at  $\delta_H$  2.34, was observed in compound (**5**). Four signals, at  $\delta_C$  74.76 (Dm), 74.76 (Dd), 75.55 (Sd), and 81.00 p.p.m. (Sm), in the <sup>13</sup>C

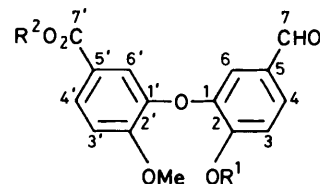
**Table 1.**  $^1\text{H}$  and  $^{13}\text{C}$  N.m.r. chemical-shift assignments of emestrin (**1**) in  $(\text{CD}_3)_2\text{SO}$ 

| Carbon | $\delta_{\text{C}}$ /p.p.m. | $\delta_{\text{H}}$  | $J(\text{H,H})/\text{Hz}$ |
|--------|-----------------------------|----------------------|---------------------------|
| 1      | 164.28 Sm                   |                      |                           |
| 3      | 75.55 Sd                    |                      |                           |
| 4      | 160.55 Sd                   |                      |                           |
| 5a     | 59.96 Dm                    | 5.672 dd             | 7.3, 2.4                  |
| 6      | 72.88 Dm                    | 4.672 ddd            | 7.3, 2.4, 2.0             |
| 7      | 107.15 Ddd                  | 4.910 dd             | 8.8, 2.0                  |
| 8      | 137.18 Ddd                  | 6.412 dd             | 8.8, 2.4                  |
| 10     | 141.68 Dm                   | 7.063 d              | 2.4                       |
| 10a    | 112.39 Sdd                  |                      |                           |
| 11     | 74.76 Dm                    | 5.466 d <sup>a</sup> | 7.1                       |
| 11a    | 81.00 Sm                    |                      |                           |
| 12     | 27.32 Q                     | 3.255 s              |                           |
| 1'     | 127.08 Sm                   |                      |                           |
| 2'     | 120.35 Dbrd                 | 7.767 d              | 2.0                       |
| 3'     | 145.31 Sdd <sup>b</sup>     |                      |                           |
| 4'     | 153.26 Sm                   |                      |                           |
| 5'     | 112.34 D                    | 6.883 d              | 8.3                       |
| 6'     | 122.66 Dm                   | 7.166 dd             | 8.3, 2.0                  |
| 7'     | 74.76 Dd                    | 4.967 d <sup>a</sup> | 4.1                       |
| 1''    | 143.44 Sdd <sup>b</sup>     |                      |                           |
| 2''    | 148.94 Sm                   |                      |                           |
| 3''    | 115.42 Dbrs                 | 7.211 d              | 8.5                       |
| 4''    | 124.92 Ddd                  | 7.576 dd             | 8.5, 2.0                  |
| 5''    | 122.15 Sdd                  |                      |                           |
| 6''    | 123.95 Dd                   | 7.377 d              | 2.0                       |
| 7''    | 164.43 Sdd                  |                      |                           |
| 8''    | 55.84 Q                     | 3.944 s              |                           |

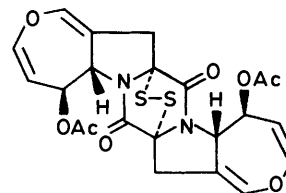
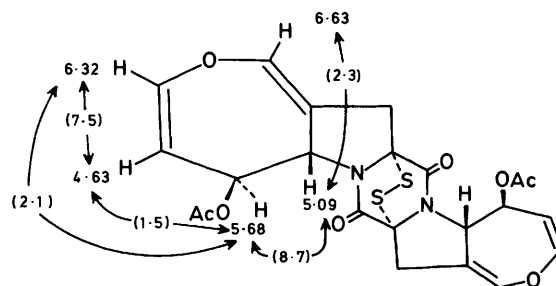
<sup>a</sup> Doublet due to C-11 hydroxy group ( $\delta$  6.254) and C-7' hydroxy group ( $\delta$  5.994). <sup>b</sup> Assignments may be reversed, but those given are the more probable.

**Figure 1.**  $^1\text{H}$  N.m.r. chemical-shift assignments of emestrin (**1**). Arrow ( $\text{H}_a \longrightarrow \text{H}_b$ ) indicates that proton b was decoupled when proton a was irradiated. Coupling constants are given in parentheses

n.m.r. spectrum of emestrin, two of which were assigned to the carbons bearing a hydroxyl group and the others to the carbons bearing nitrogens and carbonyls, disappeared and were replaced by olefinic carbon signals in the spectrum of compound (**5**). A bathochromic shift in the u.v. spectrum of compound (**5**) (360 nm) compared with that of emestrin (278 nm) or compound (**2**) (330 nm) was observed. These facts indicated that two secondary carbinols were directly attached to the carbons on both sides of a dioxopiperazine moiety.



- (**6**)  $\text{R}^1 = \text{R}^2 = \text{H}$   
 (**7**)  $\text{R}^1 = \text{R}^2 = \text{Me}$   
 (**8**)  $\text{R}^1 = \text{Me}, \text{R}^2 = \text{H}$   
 (**9**)  $\text{R}^1 = \text{Ac}, \text{R}^2 = \text{H}$

**(10)****Figure 2.**  $^1\text{H}$  N.m.r. chemical shifts of the oxepine ring of acetylaranotin (**10**). The numbers in parentheses indicate the coupling constants

Homonuclear  $^1\text{H}\{-^1\text{H}\}$  decoupling experiments of emestrin (Figure 1) and the  $^{13}\text{C}$  n.m.r. spectrum of emestrin (Table 1) confirmed the presence of two 1,2,4-trisubstituted benzene moieties. The remaining five protons at  $\delta_{\text{H}}$  7.063, 6.412, 5.672, 4.910, and 4.672 in the spectrum of emestrin were shown to couple with the six carbons at  $\delta_{\text{C}}$  141.68 (Dm), 137.18 (Ddd), 59.96 (Dm), 107.15 (Ddd), 72.88 (Dm), and 112.39 p.p.m. (Sdd). Homonuclear  $^1\text{H}\{-^1\text{H}\}$  decoupling experiments confirmed the coupling pattern as shown in Figure 1, which is similar to that of the oxepine moiety of acetylaranotin (**10**) (Figure 2), an antiviral fungal metabolite isolated from *Arachniotus aureus*.<sup>9</sup> Therefore it is likely that emestrin has a dihydro-oxepine moiety in its molecule.

Ozonolysis of compound (**5**) followed by hydrolysis gave violaceic acid (**6**) and acetylviolaceic acid (**9**), which suggested that compound (**6**) was a partial structure of emestrin and that the carboxylate was connected to the oxepine ring as an ester, and also that the aldehyde function of compound (**6**) was attached to the dioxopiperazine ring as a secondary carbinol. These results assumed the structure of emestrin as shown in structure (**1**). The assignments of  $^1\text{H}$  and  $^{13}\text{C}$  n.m.r. signals of emestrin are summarized in Table 1.

From the acidic extract of the culture filtrate was isolated violaceic acid (**6**). This compound was first isolated from *E. violacea* by Yamazaki and Maebayashi<sup>4</sup> and the structure was also proposed by them. A monomethyl ether (**8**), m.p. 200—

**Table 2.**  $^{13}\text{C}$  N.m.r. chemical shifts of violaceic acid (6) and its derivatives in  $(\text{CD}_3)_2\text{SO}$ 

| Carbon            | (6)                     | (7)          | (8)           | (9) <sup>a</sup> |
|-------------------|-------------------------|--------------|---------------|------------------|
| 1                 | 144.6 (Sm) <sup>b</sup> | 145.5 (Sdd)  | 145.58 (Sdd)  | 150.23 (Sdd)     |
| 2                 | 154.3 (Sdd)             | 155.3 (Sm)   | 155.14 (Sm)   | 145.58 (Sddd)    |
| 3                 | 117.0 (D)               | 112.9 (D)    | 112.98 (D)    | 124.41 (D)       |
| 4                 | 127.8 (Dbrd)            | 128.1 (Dbrd) | 127.97 (Dbrd) | 125.72 (Dbrd)    |
| 5                 | 128.8 (Sdd)             | 129.7 (Sdd)  | 129.70 (Sdd)  | 135.17 (Sbrdd)   |
| 6                 | 118.7 (Dd)              | 116.5 (Dbrd) | 116.76 (Dbrd) | 116.98 (Dbrd)    |
| 7                 | 190.5 (Ddd)             | 190.9 (Ddd)  | 190.88 (Ddd)  | 190.57 (Ddd)     |
| 1'                | 144.3 (Sm) <sup>b</sup> | 144.1 (Sdd)  | 144.14 (Sdd)  | 143.58 (Sdd)     |
| 2'                | 153.7 (Sm)              | 154.1 (Sm)   | 153.91 (Sm)   | 156.08 (Sm)      |
| 3'                | 112.4 (D)               | 112.9 (D)    | 112.75 (D)    | 112.27 (D)       |
| 4'                | 126.2 (Dd)              | 126.6 (Dd)   | 126.55 (Dd)   | 129.06 (Dd)      |
| 5'                | 123.2 (Sd)              | 122.2 (Sd)   | 123.39 (Sbrd) | 122.51 (Sbrd)    |
| 6'                | 118.7 (Dd)              | 119.2 (Dd)   | 119.24 (Dd)   | 123.48 (Dd)      |
| 7'                | 166.4 (Sdd)             | 165.3 (Sm)   | 166.31 (Sdd)  | 170.73 (Sdd)     |
| MeO               | 55.9 (Q)                | 56.0 (Q)     | 55.95 (Q)     | 56.10 (Q)        |
|                   |                         | 56.2 (Q)     | 56.17 (Q)     |                  |
|                   |                         | 51.9 (Q)     |               |                  |
| MeCO <sub>2</sub> |                         |              |               | 20.71 (Q)        |
|                   |                         |              |               | 168.20 (Sq)      |

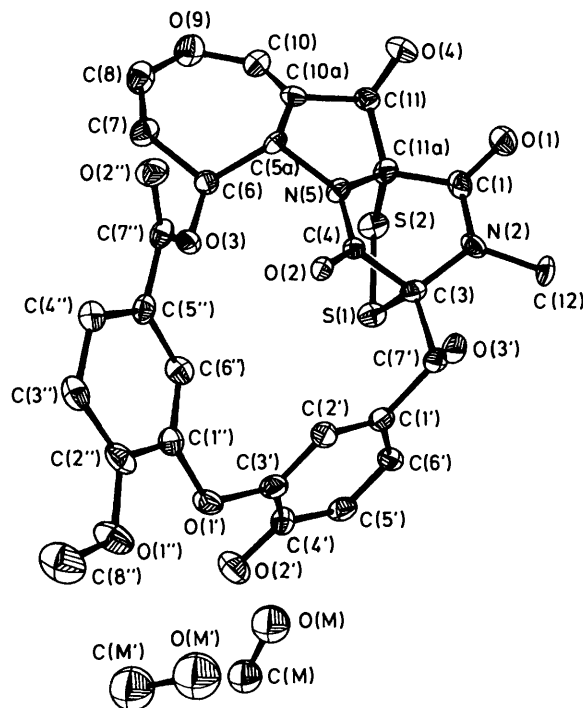
<sup>a</sup> Compound (9) was measured in  $\text{CDCl}_3$ . <sup>b</sup> Assignments may be reversed, but those given are the more probable.

201 °C, and a monoacetate (9), m.p. 168–170 °C, were prepared from compound (6) by methylation following hydrolysis and by acetylation, respectively.  $^{13}\text{C}$  N.m.r. chemical shifts of violaceic acid (6) and its derivatives are listed in Table 2. When the methoxy protons at  $\delta_{\text{H}}$  3.861 and 3.917 in compound (8) were irradiated, a 14% nuclear Overhauser enhancement (n.O.e.) of a proton signal of  $\delta_{\text{H}}$  7.268 (1 H, d,  $J$  8.4 Hz) was observed, as was a 21% n.O.e. of a proton signal of  $\delta_{\text{H}}$  7.371 (1 H, d,  $J$  8.4 Hz). The carbon signals at  $\delta_{\text{C}}$  154.1 and 155.3 p.p.m. in compound (7), which were assigned to the carbons bearing a methoxy group from the complexity of their  $^{13}\text{C}\{-^1\text{H}\}$  couplings, were observed to be changed into triplet-like signals by selective irradiation of the methoxy protons ( $\delta_{\text{H}}$  3.862–3.932). These results confirmed that the above two methoxy groups in compound (8) were undoubtedly located *para* to a carboxylic acid and *para* to an aldehyde, respectively. Then the methoxy protons at  $\delta_{\text{H}}$  3.882 in compound (9) were irradiated and an 11% n.O.e. was observed for a proton at  $\delta_{\text{H}}$  7.066 (1 H, d,  $J$  8.8 Hz). This proton was therefore assigned to the proton vicinal to the methoxy group and must be *meta* to either the carbonyl or the aldehyde. The  $^{13}\text{C}$  n.m.r. signals at  $\delta_{\text{C}}$  135.17 (Sbrdd,  $^2J_{\text{C,H}}$  24 Hz;  $\text{CHO}$ ,  $^3J_{\text{C,H}}$  9 Hz) and  $\delta$  122.51 p.p.m. (Sbrd,  $^3J_{\text{C,H}}$  9 Hz) were assigned to the carbon bearing the aldehyde (C-5) and the carbon bearing the carboxylic acid (C-5'), respectively. The signal at  $\delta_{\text{C}}$  122.51 p.p.m. was observed to change to a broad singlet on selective irradiation of the proton at  $\delta_{\text{H}}$  7.066, when a signal at  $\delta_{\text{C}}$  143.58 (Sdd,  $^3J_{\text{C,H}}$  8 and 4 Hz, C-1') was also changed into a doublet. The above result confirms that the methoxy group in violaceic acid (6) is attached to the same aromatic ring as the carboxylic acid. Thus the structure of violaceic acid should be revised to (6).

In order to determine the exact structure of emestrin including the stereochemistry, an X-ray structure analysis of the methanol adduct of emestrin was undertaken. Crystals of emestrin methanol solvate were grown in acetone–methanol solution as prisms. The relative structure of emestrin was established as shown in Figure 3 and in structure (1). Most of the bond lengths and angles are not significantly different from those expected (Tables 3 and 4), but some bond angles in the dihydro-oxepine ring are very large. The planarity between the two aromatic rings is not so good because of the formation of

**Table 3.** Bond lengths (Å) for emestrin (1) methanol solvate with estimated standard deviations in parentheses

|               |           |               |           |
|---------------|-----------|---------------|-----------|
| S(1)–S(2)     | 2.075(4)  | S(1)–C(3)     | 1.900(14) |
| S(2)–C(11a)   | 1.884(12) | C(1)–C(11a)   | 1.515(14) |
| C(1)–N(2)     | 1.333(14) | C(1)–O(1)     | 1.224(17) |
| C(3)–C(4)     | 1.560(18) | C(3)–C(7')    | 1.546(16) |
| C(3)–N(2)     | 1.456(19) | C(4)–N(5)     | 1.346(13) |
| C(4)–O(2)     | 1.218(12) | C(5a)–C(6)    | 1.561(21) |
| C(5a)–C(10a)  | 1.517(15) | C(5a)–N(5)    | 1.501(17) |
| C(6)–C(7)     | 1.517(20) | C(6)–O(3)     | 1.431(12) |
| C(7)–C(8)     | 1.291(17) | C(8)–O(9)     | 1.383(16) |
| C(10)–C(10a)  | 1.357(18) | C(10)–O(9)    | 1.368(15) |
| C(10a)–C(11)  | 1.503(15) | C(11)–C(11a)  | 1.526(16) |
| C(11)–O(4)    | 1.407(17) | C(11a)–N(5)   | 1.441(13) |
| C(12)–N(2)    | 1.466(14) | C(1')–C(2')   | 1.379(15) |
| C(1')–C(6')   | 1.412(15) | C(1')–C(7')   | 1.516(21) |
| C(2')–C(3')   | 1.392(20) | C(3')–C(4')   | 1.382(14) |
| C(3')–O(1')   | 1.387(13) | C(4')–C(5')   | 1.408(16) |
| C(4')–O(2')   | 1.355(19) | C(5')–C(6')   | 1.410(21) |
| C(7')–O(3')   | 1.427(13) | C(1'')–C(2'') | 1.385(15) |
| C(1'')–C(6'') | 1.373(14) | C(1'')–O(1'') | 1.373(16) |
| C(2'')–C(3'') | 1.403(18) | C(2'')–O(1'') | 1.362(13) |
| C(3'')–C(4'') | 1.393(14) | C(4'')–C(5'') | 1.394(15) |
| C(5'')–C(6'') | 1.385(17) | C(5'')–C(7'') | 1.487(14) |
| C(7'')–O(2'') | 1.359(12) | C(7'')–O(2'') | 1.212(12) |
| C(8'')–O(1'') | 1.423(15) | C(M)–O(M)     | 1.422(21) |
| C(M')–O(M')   | 1.368(22) |               |           |

**Figure 3.** Perspective view of the crystal structure of emestrin (1) methanol solvate with thermal ellipsoids at 30% probability

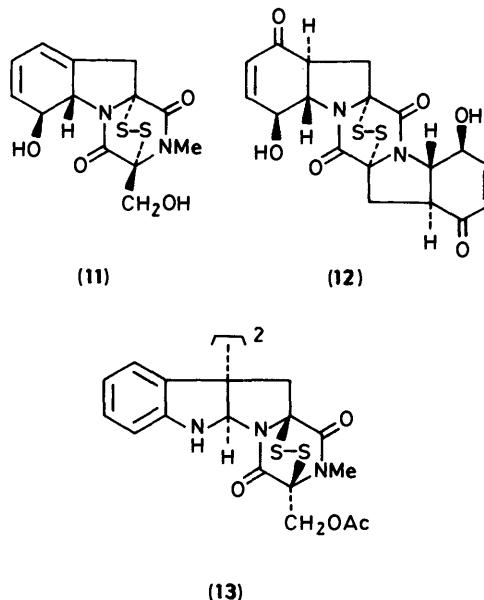
the 15-membered ring. The angle between the best planes of the aromatic ring bearing a carbonyl group and the ester is 21.8°, so conjugation of the benzoate moiety is not so good. The torsion angles in the dihydro-oxepine ring (Table 5) indicate that the coupling pattern in the  $^1\text{H}$  n.m.r. spectrum of emestrin is explicable. The molecules are packed mainly through the hydrogen bonding between the molecules of emestrin and methanol solvate in the crystals.

**Table 4.** Bond angles ( $^{\circ}$ ) for emestrin (1) methanol solvate with estimated standard deviations in parentheses

|                     |           |                    |           |
|---------------------|-----------|--------------------|-----------|
| S(2)-S(1)-C(3)      | 98.8(5)   | S(1)-S(2)-C(11a)   | 96.1(4)   |
| C(11a)-C(1)-N(2)    | 114.6(12) | C(11a)-C(1)-O(1)   | 120.2(11) |
| N(2)-C(1)-O(1)      | 125.1(9)  | S(1)-C(3)-C(4)     | 102.1(6)  |
| S(1)-C(3)-C(7')     | 106.8(6)  | S(1)-C(3)-N(2)     | 112.3(9)  |
| C(4)-C(3)-C(7')     | 115.0(9)  | C(4)-C(3)-N(2)     | 108.8(7)  |
| C(7')-C(3)-N(2)     | 111.6(8)  | C(3)-C(4)-N(5)     | 112.3(9)  |
| C(3)-C(4)-O(2)      | 122.3(8)  | N(5)-C(4)-O(2)     | 125.4(10) |
| C(6)-C(5a)-C(10a)   | 107.0(9)  | C(6)-C(5a)-N(5)    | 113.0(7)  |
| C(10a)-C(5a)-N(5)   | 100.3(8)  | C(5a)-C(6)-C(7)    | 111.5(8)  |
| C(5a)-C(6)-O(3)     | 113.6(10) | C(7)-C(6)-O(3)     | 110.4(9)  |
| C(6)-C(7)-C(8)      | 129.1(12) | C(7)-C(8)-O(9)     | 129.1(12) |
| C(10a)-C(10)-O(9)   | 128.0(13) | C(5a)-C(10a)-C(10) | 134.1(10) |
| C(5a)-C(10a)-C(11)  | 110.1(10) | C(10)-C(10a)-C(11) | 115.3(11) |
| C(10a)-C(11)-C(11a) | 101.4(12) | C(10a)-C(11)-O(4)  | 108.1(12) |
| C(11a)-C(11)-O(4)   | 109.9(9)  | S(2)-C(11a)-C(1)   | 102.3(11) |
| S(2)-C(11a)-C(11)   | 109.5(7)  | S(2)-C(11a)-N(5)   | 110.9(9)  |
| C(1)-C(11a)-C(11)   | 116.3(12) | C(1)-C(11a)-N(5)   | 112.7(9)  |
| C(11)-C(11a)-N(5)   | 105.2(10) | C(2')-C(1')-C(6')  | 120.0(11) |
| C(2')-C(1')-C(7')   | 122.3(10) | C(6')-C(1')-C(7')  | 117.6(10) |
| C(1')-C(2')-C(3')   | 119.9(10) | C(2')-C(3')-C(4')  | 121.5(10) |
| C(2')-C(3')-O(1')   | 121.3(10) | C(4')-C(3')-O(1')  | 117.1(11) |
| C(3')-C(4')-C(5')   | 118.9(12) | C(3')-C(4')-O(2')  | 119.1(10) |
| C(5')-C(4')-O(2')   | 121.9(10) | C(4')-C(5')-C(6')  | 120.0(11) |
| C(1')-C(6')-C(5')   | 119.2(11) | C(3)-C(7')-C(1')   | 113.4(8)  |
| C(3)-C(7')-O(3')    | 108.0(8)  | C(1')-C(7')-O(3')  | 113.7(9)  |
| C(2')-C(1')-C(6')   | 121.2(11) | C(2')-C(1')-O(1')  | 116.3(9)  |
| C(6')-C(1')-O(1')   | 122.5(10) | C(1')-C(2')-C(3')  | 119.6(10) |
| C(1')-C(2')-O(1')   | 116.6(10) | C(3')-C(2')-O(1')  | 123.8(10) |
| C(2')-C(3')-C(4')   | 119.6(10) | C(3')-C(4')-C(5')  | 118.9(10) |
| C(4')-C(5')-C(6')   | 121.5(10) | C(4')-C(5')-C(7')  | 119.0(10) |
| C(6')-C(5')-C(7')   | 119.5(9)  | C(1')-C(6')-C(5')  | 118.9(10) |
| C(5')-C(7')-O(3)    | 110.3(8)  | C(5')-C(7')-O(2')  | 125.6(9)  |
| O(3)-C(7')-O(2')    | 124.1(9)  | C(1)-N(2)-C(3)     | 117.2(9)  |
| C(1)-N(2)-C(12)     | 120.1(11) | C(3)-N(2)-C(12)    | 121.9(9)  |
| C(4)-N(5)-C(5a)     | 129.0(9)  | C(4)-N(5)-C(11a)   | 117.9(9)  |
| C(5a)-N(5)-C(11a)   | 113.1(8)  | C(6)-O(3)-C(7')    | 120.3(7)  |
| C(8)-O(9)-C(10)     | 124.5(11) | C(3')-O(1')-C(1')  | 117.4(10) |
| C(2')-O(1')-C(8')   | 118.0(10) |                    |           |

**Table 5.** Torsion angles ( $^{\circ}$ ) within the dihydro-oxepine ring with standard deviations in parentheses

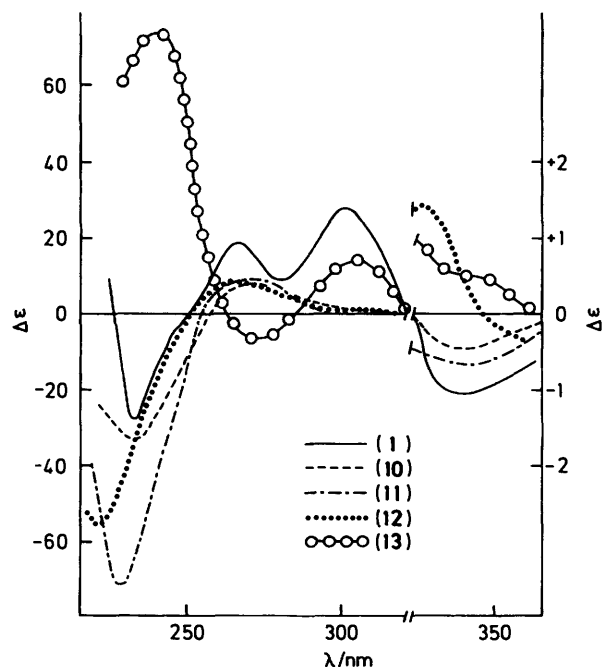
|                         |            |
|-------------------------|------------|
| C(10a)-C(5a)-C(6)-C(7)  | -67.0(1.0) |
| C(5a)-C(6)-C(7)-C(8)    | 55.4(1.6)  |
| C(6)-C(7)-C(8)-O(9)     | 0.1(2.1)   |
| C(7)-C(8)-O(9)-C(10)    | -19.0(1.9) |
| C(8)-O(9)-C(10)-C(10a)  | -9.6(1.9)  |
| O(9)-C(10)-C(10a)-C(5a) | 12.2(2.2)  |
| C(10)-C(10a)-C(5a)-C(6) | 32.9(1.6)  |



The c.d. spectra of the epidithiodioxopiperazine derivatives are known to show maxima at 235, 270, 310, and 340 nm from the theoretical analysis.<sup>9</sup> Comparison of the c.d. curve of emestrin with those of the fungal epidithiodioxopiperazines acetylaranotin (10), gliotoxin (11),<sup>9</sup> epicorazine A (12) from *Epicoccum nigrum*,<sup>10</sup> and di-O-acetylchaetocin (13) from *Chaetomium minutum*,<sup>11</sup> are shown in Figure 4. Thus it is now clear that compounds (10), (11), and (12) have the same configuration about the epidithiodioxopiperazine ring as does emestrin, and that compound (13) has the opposite configuration. The positive Cotton effect observed at 301 nm for emestrin and that observed at 297 nm in compound (2) were due to the benzoate moiety. Emestrin must therefore have the 3*R*,11*aR* configuration and consequently the absolute structure was determined as (1).

Emestrin (1) is a fungal metabolite having a characteristic macrocyclic epidithiodioxopiperazine structure, which is biogenetically derived from the combination of one molecule of benzoic acid with the epidithiodioxopiperazine structure formed from two molecules of phenylalanine. It has a new skeleton involving a 15-membered ring. This is the first example of a dioxopiperazine derivative which possesses a macrocyclic ring. Furthermore, emestrin (1) was shown to be the same compound as that recently isolated as a mycotoxin from *Emericella quadrilineata*, *E. acristata*, and *E. parvathecica*, and designated EQ-1, by Maebayashi *et al.*<sup>12</sup>

A preliminary test showed that the mycelial chloroform extract of *E. striata* possessed antifungal activity. Emestrin

**Figure 4.** C.d. spectra of emestrin (1) and related compounds

**Table 6.** Antifungal activity of emestrin (1)

| Amount of (1)<br>( $\mu\text{g}/\text{disc}$ ) | Test organism               |                        |
|--|-----------------------------|------------------------|
|  | <i>Penicillium expansum</i> | <i>Gibberella zeae</i> |
| 100  | 29 min <sup>a</sup>         | 30 mm                  |
| 25   | 29                          | 30                     |
| 10   | 25                          | 30                     |
| 2.5  | 19                          | 19                     |
| 1.0  | 10                          | 12                     |
| 0.25   | — <sup>b</sup>              | —                      |

<sup>a</sup> Diameter of inhibition circle. <sup>b</sup> Dash (—) means no inhibition.

(1) inhibited the growth of *Gibberella zeae* and *Penicillium expansum* at a concentration of 1.0  $\mu\text{g}$  per disc (Table 6). No other metabolites, e.g. emericellin, sterigmatocystin, or violaceic acid (6), with antifungal activity could be detected. The minimum inhibitory concentrations (MIC) of emestrin (1) against *G. zeae* and *P. expansum* were 10 and 2.5  $\mu\text{g ml}^{-1}$ , respectively, as determined by the cylinder-agar plate assay.

### Experimental

M.p.s were determined on a Yanagimoto micro-melting point apparatus and are uncorrected. Optical rotations were measured with a JASCO DIP-181 spectrometer. Electron impact (EI) and FD mass spectra were taken with a JEOL JMS-D 300 spectrometer. U.v. spectra and i.r. spectra were recorded on a Hitachi 124 spectrophotometer and a Hitachi 215 spectrophotometer, respectively. <sup>1</sup>H (99.60 MHz) and <sup>13</sup>C (25.05 MHz) N.m.r. spectra were recorded on a JEOL JNM-FX 100 spectrometer, while <sup>1</sup>H (399.65 MHz) and <sup>13</sup>C (100.40 MHz) n.m.r. spectra were taken with a JEOL JNM-GX 400 spectrometer, using tetramethylsilane as internal standard. The coupling patterns are indicated as follows: singlet = S or s, doublet = D or d, triplet = T or t, quartet = Q or q, multiplet = m, and broad = br. Capital letters refer to the pattern resulting from directly bonded coupling (<sup>1</sup>J<sub>C,H</sub>). C.d. curves were determined on a JASCO J-40 spectrophotometer. Column chromatography was performed using Kieselgel 60 (Art. 7734; Merck). T.l.c. was conducted on precoated Kieselgel 60 F<sub>254</sub> (Art. 5715; Merck). Spots on t.l.c. plates were detected by their absorption under u.v. light, and/or with iodine vapour.

**Isolation of Emestrin (1) and Other Metabolites.**—*Emericella striata*, strain 80-NE-22, was cultivated at 30 °C for 3 weeks in Czapek-Dox medium. The culture filtrate (50 l) was extracted with dichloromethane at pH 2 to obtain violaceic acid (6) (840 mg) after purification with column chromatography [benzene-acetone (5:1, v/v)]. The dried mycelia (660 g) were pulverized and extracted with acetone at room temperature. The residue obtained on evaporation was treated with chloroform, and the chloroform solution was separated from insoluble material, dried (Na<sub>2</sub>SO<sub>4</sub>) and concentrated under reduced pressure. The residue (26 g) was chromatographed on silica gel with benzene-acetone (100:1, v/v) to obtain emericellin (320 mg) and sterigmatocystin (12 mg), with benzene-acetone (50:1, v/v) to give ergosterol (1.5 g), and with benzene-acetone (10:1, v/v) to obtain emestrin (1) (1.06 g).

**Emestrin (1)** was obtained as prisms (from acetone), m.p. 233–236 °C (decomp.); [ $\alpha$ ]<sub>D</sub><sup>15</sup> +184° (c 0.23 in CHCl<sub>3</sub>) (Found: C, 54.3; H, 3.6; N, 4.7. C<sub>27</sub>H<sub>22</sub>N<sub>2</sub>O<sub>10</sub>S<sub>2</sub> requires C, 54.2; H, 3.7; N, 4.7%; *m/z* 598 (*M*<sup>+</sup>, 100%, FD), 534 [(*M* – S<sub>2</sub>)<sup>+</sup>, 8], 516 (12), and 288 (5); *m/z* 534 [(*M* – S<sub>2</sub>)<sup>+</sup>, 2%, EI], 287 (100), and 64 (S<sub>2</sub><sup>+</sup>, 16);  $\lambda_{\text{max}}$  (MeOH) 230 (log  $\epsilon$  4.44), 262 (4.19), and 278 nm (3.96);  $\nu_{\text{max}}$  (KBr) 3 400 (OH), 1 710 (CO<sub>2</sub>), 1 680, 1 660

[C(=O)N], and 1 610 cm<sup>-1</sup>;  $\delta_{\text{H}}$  [(CD<sub>3</sub>)<sub>2</sub>SO] 3.255 (3 H, s, NMe), 3.944 (3 H, s, OMe), 4.672 (1 H, ddd, *J* 7.3, 2.4, and 2.0 Hz, 6-H), 4.910 (1 H, dd, *J* 8.8 and 2.0 Hz, 7-H), 4.967 (1 H, d, *J* 4.1 Hz, 7'-H), 5.466 (1 H, d, *J* 7.1 Hz, 11-H), 5.672 (1 H, dd, *J* 7.3 and 2.4 Hz, 5a-H), 5.994 (1 H, d, *J* 4.1 Hz, 7'-OH), 6.254 (1 H, d, *J* 7.1 Hz, 11-OH), 6.412 (1 H, dd, *J* 8.8 and 2.4 Hz, 8-H), 6.883 (1 H, d, *J* 8.3 Hz, 5'-H), 7.063 (1 H, d, *J* 2.4 Hz, 10-H), 7.166 (1 H, dd, *J* 8.3 and 2.0 Hz, 6'-H), 7.211 (1 H, d, *J* 8.5 Hz, 3'-H), 7.377 (1 H, d, *J* 2.0 Hz, 6''-H), 7.576 (1 H, dd, *J* 8.5 and 2.0 Hz, 4''-H), 7.767 (1 H, d, *J* 2.0 Hz, 2'-H), and 9.739 (1 H, s, 4'-OH); c.d. (c 5.7  $\times 10^{-5}$  in MeOH) [ $\theta$ ]<sub>233</sub> -9.50  $\times 10^4$ , [ $\theta$ ]<sub>266</sub> +6.24  $\times 10^4$ , [ $\theta$ ]<sub>301</sub> +9.32  $\times 10^4$ , and [ $\theta$ ]<sub>338</sub> -0.38  $\times 10^4$ .

**Acetylation of Emestrin (1).**—Emestrin (1) (200 mg) was dissolved in pyridine (1.5 ml) containing acetic anhydride (1 ml) and the solution was kept overnight at room temperature. The mixture was poured into ice-water and extracted with chloroform. The evaporated extract afforded a residue which was crystallized from methanol to give *triacetylemestrin* (2) (205 mg) as needles, m.p. 215–217 °C (decomp.), [ $\alpha$ ]<sub>D</sub><sup>15</sup> -27.4° (c 1.41 in CHCl<sub>3</sub>) (Found: C, 54.8; H, 4.0; N, 3.6. C<sub>33</sub>H<sub>28</sub>N<sub>2</sub>O<sub>13</sub>S<sub>2</sub> requires C, 54.7; H, 3.9; N, 3.9%;  $\lambda_{\text{max}}$  (MeOH) 257 (log  $\epsilon$  4.21), 263sh (4.21), 288sh (3.77), and 330 nm (3.20);  $\nu_{\text{max}}$  (KBr) 1 750, 1 720 (CO<sub>2</sub>), 1 695, and 1 680 cm<sup>-1</sup> [C(=O)N];  $\delta_{\text{H}}$  [(CD<sub>3</sub>)<sub>2</sub>SO] 2.02 (3 H, s, OAc), 2.10 (3 H, s, OAc), 2.29 (1 H, s, 4'-OAc), 3.11 (3 H, s, NMe), 3.94 (3 H, s, 2''-OMe), 4.74 (1 H, ddd, *J* 7.3, 2.4, and 2.2 Hz, 6-H), 5.01 (1 H, dd, *J* 8.3 and 2.2 Hz, 7-H), 5.68 (1 H, dd, *J* 7.3 and 2.4 Hz, 5a-H), 6.44 (1 H, s, 7'-H), 6.46 (1 H, dd, *J* 8.3 and 2.4 Hz, 8-H), 6.63 (1 H, s, 11-H), 7.10 (1 H, d, *J* 2.4 Hz, 10-H), 7.22 (1 H, d, *J* 8.3 Hz, 3'-H), 7.30 (1 H, d, *J* 8.3 Hz, 5'-H), 7.33 (1 H, d, *J* 2.0 Hz, 6''-H), 7.63 (1 H, dd, *J* 8.3 and 2.0 Hz, 4''-H), 7.69 (1 H, dd, *J* 8.3 and 2.0 Hz, 6'-H), and 7.75 (1 H, d, *J* 2.0 Hz, 2'-H);  $\delta_{\text{C}}$  (CDCl<sub>3</sub>) 2.08 (3 H, s, OAc), 2.10 (3 H, s, OAc), 2.34 (3 H, s, 4'-OAc), 3.10 (3 H, s, NMe), 3.99 (3 H, s, OMe), 4.78 (1 H, ddd, *J* 7.3, 2.4, and 2.2 Hz, 6-H), 4.93 (1 H, dd, *J* 8.3 and 2.2 Hz, 7-H), 5.91 (1 H, dd, *J* 7.3 and 2.4 Hz, 5a-H), 6.18 (1 H, s, 7'-H), 6.29 (1 H, dd, *J* 8.3 and 2.4 Hz, 8-H), 6.47 (1 H, s, 11-H), 7.00 (1 H, d, *J* 8.5 Hz), 7.02 (1 H, d, *J* 2.4 Hz, 10-H), 7.14 (1 H, d, *J* 8.5 Hz), 7.32 (1 H, dd, *J* 8.5 and 2.0 Hz), 7.49 (1 H, d, *J* 2.0 Hz), 7.76 (1 H, dd, *J* 8.5 and 2.0 Hz), and 7.90 (1 H, d, *J* 2.0 Hz);  $\delta_{\text{C}}$  (CDCl<sub>3</sub>) 20.7 (Q, OCOMe), 20.9 (Q, 2  $\times$  OCOMe), 28.1 (Q, NMe), 56.2 (Q, 2''-OMe), 60.8 (D, C-5a), 73.7 (D), 74.4 (D), 74.9 (D), 75.1 (S, C-3), 80.4 (S, C-11a), 107.7 (D), 109.2 (S), 112.0 (D), 121.5 (D), 122.5 (D), 122.5 (S), 123.3 (D), 125.2 (D), 125.5 (D), 130.2 (S), 137.0 (D), 143.3 (S), 143.9 (D), 145.3 (S), 149.0 (S), 153.8 (S), 159.2 [S, C(=O)N], 163.2 [S, C(=O)N], 165.1 (S, CO<sub>2</sub>), 168.4 (S, OCOMe), 169.1 (S, OCOMe), and 169.4 p.p.m. (S, OCOMe); c.d. (c 6.8  $\times 10^{-5}$  in MeOH) [ $\theta$ ]<sub>233</sub> -5.8  $\times 10^4$ , [ $\theta$ ]<sub>268</sub> +2.0  $\times 10^4$ , [ $\theta$ ]<sub>297</sub> +4.7  $\times 10^4$ , and [ $\theta$ ]<sub>336</sub> -0.2  $\times 10^4$ .

**Reductive Methylation of Emestrin (1) with Sodium Borohydride and Iodomethane.**—Sodium borohydride (25 mg) was added during 30 min to a stirred solution of emestrin (1) (131 mg) in a mixture of pyridine (2 ml), methanol (2 ml), and iodomethane (1 ml). After addition of further iodomethane (1 ml), the reaction mixture was stirred at room temperature for 3 h, poured into water, acidified, and extracted with chloroform. The extract was evaporated and the residue was purified on a silica gel column with chloroform-methanol (100:1, v/v) as solvent, to yield *didethiobis(methylthio)emestrin* (3) (52 mg) as a white crystalline powder (from benzene-ether), m.p. 184–186 °C (Found: C, 55.1; H, 4.5; N, 4.2. C<sub>29</sub>H<sub>28</sub>N<sub>2</sub>O<sub>10</sub>S<sub>2</sub> requires C, 55.4; H, 4.5; N, 4.5%;  $\lambda_{\text{max}}$  (MeOH) 259 (log  $\epsilon$  4.11) and 287 nm (3.67);  $\nu_{\text{max}}$  (KBr) 3 450 (OH), 1 710 (CO<sub>2</sub>), 1 670, 1 650 [C(=O)N], and 1 600 cm<sup>-1</sup>;  $\delta_{\text{H}}$  [(CD<sub>3</sub>)<sub>2</sub>SO] 1.907 (3 H, s, SMe), 2.275 (3 H, s, SMe), 3.165 (3 H, s, NMe), 3.929 (3 H, s, OMe), 4.809 (1 H, d, *J* 4.4 Hz, 7'-H), 4.911 (1 H, dd, *J* 8.0 and 2.2 Hz,

7-H), 5.079 (1 H, dd,  $J$  8.0 and 2.4 Hz, 5a-H), 5.222 (1 H, ddd,  $J$  8.0, 2.4, and 2.2 Hz, 6-H), 5.318 (1 H, br d,  $J$  7.6 Hz, 11-H), 5.948 (1 H, d,  $J$  4.4 Hz, 7'-OH), 5.963 (1 H, d,  $J$  7.6 Hz, 11-OH), 6.450 (1 H, dd,  $J$  8.0 and 2.4 Hz, 8-H), 6.857 (1 H, d,  $J$  8.6 Hz), 7.034 (1 H, d,  $J$  2.4 Hz, 10-H), 7.170 (1 H, d,  $J$  8.6 Hz), 7.241 (1 H, dd,  $J$  8.6 and 2.0 Hz), 7.630 (1 H, dd,  $J$  8.6 and 2.0 Hz), 7.703 (1 H, d,  $J$  2.0 Hz), 8.296 (1 H, d,  $J$  2.0 Hz), and 9.132 (1 H, s, 4'-OH).

**Reductive Methylation of the Triacetate (2) with Sodium Borohydride and Iodomethane.**—Sodium borohydride (50 mg) was added during 30 min to a stirred solution of compound (2) (250 mg) in a mixture of tetrahydrofuran (THF) (3 ml), pyridine (1 ml), and iodomethane (1.5 ml). After addition of further iodomethane (1 ml), the reaction mixture was stirred at room temperature for 3 h, poured into water, acidified with 2M-HCl, and extracted with chloroform. The extract was evaporated and the residue was purified on a silica gel column with benzene-acetone (20:1, v/v) as solvent to obtain *triacetylalidethiobis(methylthio)emestrin (4)* (107 mg) as needles (from EtOH), m.p. 225–227 °C;  $[\alpha]_D^{15} + 62.3$  ( $c$  0.79 in  $\text{CHCl}_3$ ) (Found: C, 55.9; H, 4.5; N, 3.6.  $\text{C}_{35}\text{H}_{34}\text{N}_2\text{O}_{13}\text{S}_2$  requires C, 55.7; H, 4.5; N, 3.7%);  $\lambda_{\text{max}}$ (EtOH) 210 (log  $\epsilon$  4.76), 261 (4.30), and 291 nm (3.73);  $\nu_{\text{max}}$ (KBr) 1 745, 1 727, 1 710 ( $\text{CO}_2$ ) and 1 660  $\text{cm}^{-1}$  [ $\text{C}(\text{=O})\text{N}$ ];  $\delta_{\text{H}}$ ( $\text{CDCl}_3$ ) 1.50 (3 H, s, SMe), 1.98 (3 H, s, SMe), 2.18 (3 H, s, OAc), 2.31 (3 H, s, OAc), 2.34 (3 H, s, OAc), 3.15 (3 H, s, NMe), 3.98 (3 H, s, OMe), 4.98 (1 H, br d,  $J$  7.8 Hz, 7-H), 5.35 (2 H, br s, 5a- and 6-H), 5.93 (1 H, s, 7'-H), 6.33 (1 H, dd,  $J$  7.8 and 1.7 Hz, 8-H), 6.51 (1 H, s, 11-H), 6.96 (1 H, d,  $J$  8.5 Hz), 7.04 (1 H, d,  $J$  2.0 Hz, 10-H), 7.05 (1 H, d,  $J$  8.5 Hz), 7.25 (1 H, dd,  $J$  8.5 and 2.2 Hz), 7.76 (1 H, dd,  $J$  8.5 and 2.2 Hz), 8.08 (1 H, d,  $J$  2.2 Hz), and 8.80 (1 H, d,  $J$  2.2 Hz);  $\delta_{\text{C}}$ ( $\text{CDCl}_3$ ) 14.5 (Q, SMe), 14.8 (Q, SMe), 20.6 (Q, OCOMe), 21.0 (Q, 2  $\times$  OCOMe), 30.1 (Q, NMe), 56.3 (Q, 2'-OMe), 58.4 (D, C-5a), 71.4 (S, C-3 or -11a), 72.5 (S, C-11a or -3), 75.0 (D), 75.7 (D), 78.9 (D), 107.9 (D), 107.9 (S), 112.0 (D), 120.9 (D), 122.2 (D), 122.2 (S), 126.7 (D), 127.1 (D), 127.3 (D), 133.6 (S), 139.1 (D), 142.6 (S), 142.8 (D), 146.6 (S), 146.7 (S), 154.5 (S), 162.4 [S,  $\text{C}(\text{=O})\text{N}$ ], 165.3 [S,  $\text{C}(\text{=O})\text{N}$  or  $\text{CO}_2$ ], 165.5 [ $\text{CO}_2$  or  $\text{C}(\text{=O})\text{N}$ ], 168.4 (S, OCOMe), 169.2 (S, OCOMe), and 169.8 p.p.m. (S, OCOMe); c.d. ( $c$   $5.6 \times 10^{-5}$  in MeOH)  $[\theta]_{244}^{244} + 5.77 \times 10^4$ ,  $[\theta]_{270}^{270} + 1.87 \times 10^4$ , and  $[\theta]_{295}^{295} - 2.52 \times 10^4$ .

**Desulphurization of the Triacetate (2) with Raney Nickel.**—Raney nickel (1.5 g) and water (2 ml) were added to a solution of the triacetate (2) (150 mg) in acetone (10 ml). The mixture was heated at 50 °C for 2 h, and then the catalyst was separated by filtration and washed successively with hot acetone and hot chloroform. The combined filtrate and washings were concentrated under reduced pressure to a volume of 5 ml and extracted with chloroform after addition of water. The extract was evaporated and the residue was chromatographed on silica gel with benzene-acetone (25:1, v/v) to afford *acetyldianhydrodithioemestrin (5)* (84 mg) as yellowish plates (from chloroform-acetone), m.p. 282–285 °C,  $[\alpha]_D^{15} - 420$  ( $c$  0.87 in chloroform) (Found: C, 63.9; H, 4.0; N, 5.0.  $\text{C}_{29}\text{H}_{22}\text{N}_2\text{O}_9$  requires C, 64.2; H, 4.1; N, 5.2%);  $m/z$  542 ( $M^+$ , 4%, EI), 500 [ $(M - \text{C}_2\text{H}_2\text{O})^+$ , 9], and 55 (100);  $\lambda_{\text{max}}$ (EtOH) 239 (log  $\epsilon$  4.31), 263 (4.25), 294 (4.00), and 360 nm (4.19);  $\nu_{\text{max}}$ (KBr) 1 740, 1 710 ( $\text{CO}_2$ ), 1 690, 1 670 [ $\text{C}(\text{=O})\text{N}$ ], and 1 610  $\text{cm}^{-1}$ ;  $\delta_{\text{H}}$ ( $\text{CDCl}_3$ ) 2.34 (3 H, s, OAc), 3.35 (3 H, s, NMe), 3.94 (3 H, s, OMe), 4.95 (1 H, dd,  $J$  8.2 and 1.9 Hz, 7-H), 5.04 (1 H, ddd,  $J$  7.6, 2.2, and 1.9 Hz, 6-H), 5.47 (1 H, dd,  $J$  7.6 and 2.4 Hz, 5a-H), 6.30 (1 H, dd,  $J$  8.2 and 2.2 Hz, 8-H), 6.52 (1 H, s, 11-H), 6.67 (1 H, s, 7'-H), 6.79 (1 H, d,  $J$  2.4 Hz, 10-H), 6.94 (1 H, dd,  $J$  8.2 and 2.0 Hz), 7.00 (1 H, d,  $J$  8.5 Hz), 7.08 (1 H, d,  $J$  2.0 Hz), 7.17 (1 H, d,  $J$  8.2 Hz), 7.87 (1 H, dd,  $J$  8.5 and 2.0 Hz), and 8.12 (1 H, d,  $J$  2.0 Hz);  $\delta_{\text{C}}$ ( $\text{CDCl}_3$ ) 20.66 (Q, OCOMe), 29.92 (Q, NMe), 56.28 (Q, OMe), 63.40 (D, C-5a), 71.18 (D, C-6), 107.89 (D), 112.40 (D), 116.91 (D), 119.17 (D), 119.24 (S), 119.48 (D), 120.96 (D), 121.03

(S), 121.60 (D), 123.21 (D), 126.32 (D), 132.81 (S), 132.99 (S), 134.69 (S), 138.14 (D), 139.59 (D), 140.58 (S), 144.41 (S), 146.11 (S), 154.41 (S), 154.56 [S,  $\text{C}(\text{=O})\text{N}$ ], 154.67 [S,  $\text{C}(\text{=O})\text{N}$ ], 165.58 (S,  $\text{CO}_2$ ), and 168.73 (S, 4'-OCOMe); c.d. ( $c$   $3.7 \times 10^{-5}$  in MeOH)  $[\theta]_{249}^{249} + 3.13 \times 10^4$ ,  $[\theta]_{266}^{266} + 2.44 \times 10^4$ ,  $[\theta]_{297}^{297} - 1.39 \times 10^4$ , and  $[\theta]_{358}^{358} - 1.30 \times 10^4$ .

**Hydrolysis of the Triacetate (2) with Potassium Carbonate.**—Triacetate (2) (50 mg) was refluxed for 2 h in a mixture of THF (10 ml) and 10% aqueous potassium carbonate (10 ml). The reaction mixture was concentrated under reduced pressure to a volume of 10 ml and extracted with chloroform. The extract was evaporated and the residue was chromatographed on silica gel to give crystals (from acetone) (12 mg), m.p. 282–284 °C. This compound was identified as acetyldianhydrodithioemestrin (5) by the comparison (t.l.c.,  $^1\text{H}$  n.m.r.) and also by mixed m.p.

**Ozonolysis of Compound (5) followed by Hydrolysis.**—Ozone was introduced into a solution of compound (5) (50 mg) in chloroform at  $-50$  °C and then the mixture was stirred for 1 h at room temperature after addition of zinc (10 mg) and acetic acid (1 ml). The zinc was separated by filtration and the filtrate was refluxed for 1 h with 10% aqueous potassium carbonate (5 ml). The mixture was acidified and extracted with chloroform, and the extract was dried ( $\text{Na}_2\text{SO}_4$ ) and evaporated. The residue was chromatographed on silica gel (eluant benzene-acetone 5:1) to afford violaceic acid (6) (10 mg) as pale yellow needles (from acetone), m.p. 223–225 °C, and acetylviolaceic acid (9) (2 mg) as a pale yellow powder, m.p. 198–200 °C. These two compounds were identified with those from the acidic extract of the culture filtrate by comparison of t.l.c. behaviour,  $^1\text{H}$  n.m.r. spectra, and mixed m.p.

**Isolated Violaceic Acid (6).**—This compound was obtained as pale yellow needles (from acetone), m.p. 223–225 °C;  $m/z$  288 ( $M^+$ , EI);  $\lambda_{\text{max}}$ (MeOH) 232 (log  $\epsilon$  4.41), 253 (4.24), 276 (4.18), 283 (4.14), and 310 nm (3.85);  $\nu_{\text{max}}$ (KBr) 3 420 (OH), 2 950–2 400 ( $\text{CO}_2\text{H}$ ), 1 690, 1 680 ( $\text{CO}_2\text{H}$ ), and 1 600  $\text{cm}^{-1}$ ;  $\delta_{\text{H}}$ [( $\text{CD}_3$ ) $_2\text{SO}$ ] 3.894 (3 H, s, OMe), 7.164 (1 H, d,  $J$  8.2 Hz), 7.263 (1 H, d,  $J$  8.7 Hz), 7.283 (1 H, d,  $J$  1.9 Hz), 7.315 (1 H, d,  $J$  2.2 Hz), 7.639 (1 H, dd,  $J$  8.2 and 2.2 Hz), 7.779 (1 H, dd,  $J$  8.7 and 1.9 Hz), 9.760 (1 H, s, CHO), and 10.85 (1 H, s, 2-OH). Compound (6) was identical with violaceic acid from *E. violacea* by t.l.c., i.r. and  $^{13}\text{C}$  n.m.r. spectra, and mixed m.p.

**Methylation of Violaceic Acid (6).**—Acid (6) (250 mg) was methylated in stirred ether-acetone with diazomethane for 15 min. The evaporated reaction mixture afforded a residue which was chromatographed on silica gel with benzene-acetone (50:1, v/v) to give the dimethyl ether methyl ester (7) (220 mg) as a pale yellow crystalline powder (from MeOH), m.p. 120–122 °C;  $\delta_{\text{H}}$ [( $\text{CD}_3$ ) $_2\text{SO}$ ] 3.862 (3 H, s, OMe), 3.917 (3 H, s, OMe), 3.932 (3 H, s, OMe), 7.228 (1 H, d,  $J$  1.8 Hz), 7.260 (1 H, d,  $J$  8.4 Hz), 7.342 (1 H, d,  $J$  1.8 Hz), 7.358 (1 H, d,  $J$  8.4 Hz), 7.747 (1 H, dd,  $J$  8.4 and 1.8 Hz), 7.797 (1 H, dd,  $J$  8.4 and 1.8 Hz), and 9.805 (1 H, s, CHO).

**Hydrolysis of Compound (7).**—Compound (7) (100 mg) was refluxed in a mixture of methanol (15 ml) and 10% aqueous potassium carbonate (15 ml) for 1 h. After removal of methanol, the reaction mixture was acidified and extracted with chloroform. The extract was evaporated and the residue was chromatographed on silica gel (eluant benzene-acetone 10:1) to give the dimethoxy acid (8) (84 mg) as pale yellow plates (from MeOH), m.p. 200–201 °C;  $\delta_{\text{H}}$ [( $\text{CD}_3$ ) $_2\text{SO}$ ] 3.861 (3 H, s, OMe), 3.917 (3 H, s, OMe), 7.212 (1 H, d,  $J$  1.8 Hz), 7.268 (1 H, d,  $J$  8.4 Hz), 7.315 (1 H, d,  $J$  1.8 Hz), 7.371 (1 H, d,  $J$  8.4 Hz), 7.755

(1 H, dd,  $J$  8.4 and 1.8 Hz), 7.788 (1 H, dd,  $J$  8.4 and 1.8 Hz), and 9.804 (1 H, s, CHO).

**Acetylation of Violaceic Acid (6).**—Violaceic acid (6) (100 mg) was acetylated with acetic anhydride (0.5 ml) and pyridine (0.5 ml) overnight at room temperature. The reaction mixture was poured into ice–water and extracted with ether. The extract was evaporated and the residue was separated by preparative t.l.c. with benzene–acetone–acetic acid (5:1:0.01, v/v) as the developing solvent to afford *acetylviolaceic acid* (9) (68 mg) as plates (from acetone–hexane, m.p. 168–170 °C (decomp.);  $m/z$  330 ( $M^+$ , 100%, EI);  $\nu_{\max}$  (KBr) 2 950–2 400 ( $\text{CO}_2\text{H}$ ), 1 760 (OAc), 1 690 ( $\text{CO}_2\text{H}$ ), and 1 600  $\text{cm}^{-1}$  (Found: C, 60.4; H, 4.2.  $\text{C}_{17}\text{H}_{14}\text{O}_7 \cdot 0.5\text{H}_2\text{O}$  requires C, 60.2; H, 4.5%);  $\delta_{\text{H}}$  ( $\text{CDCl}_3$ ) 2.313 (3 H, s, OAc), 3.882 (3 H, s, OMe), 7.066 (1 H, d,  $J$  8.8 Hz, 3'-H), 7.244 (1 H, d,  $J$  1.8 Hz, 6-H), 7.336 (1 H, d,  $J$  8.1 Hz, 3-H), 7.606 (1 H, dd,  $J$  8.1 and 1.8 Hz, 4-H), 7.773 (1 H, d,  $J$  2.1 Hz, 6'-H), 7.993 (1 H, dd,  $J$  8.8 and 2.1 Hz, 4'-H), and 9.861 (1 H, s, CHO).

**Structure Determination of Emestrin (1) Methanol Solvate by X-Ray Diffraction.**—Emestrin (1) was grown from a mixture of methanol–acetone to give its methanol solvate as prisms, m.p. 229–232 °C (Found: C, 53.6; H, 4.4; N, 4.0.  $\text{C}_{27}\text{H}_{22}\text{N}_2\text{O}_{10}\text{S}_2 \cdot \text{CH}_4\text{O}$  requires C, 53.3; H, 4.2; N, 4.4%). Diffraction intensities were collected from a crystal of dimensions 0.65 × 0.25 × 0.20 mm on a Rigaku AFC-5 FOS four-circle diffractometer. Of the total 2 758 reflections (complete for  $2\theta \leq 50^\circ$ ), 1 660 satisfied the criterion  $F > 2.5\sigma(F)$  and only these were used in the solution and refinement of the structure.

**Crystal data.**— $\text{C}_{27}\text{H}_{22}\text{N}_2\text{O}_{10}\text{S}_2 \cdot 2\text{CH}_4\text{O}$ ,  $M = 662.7$ , monoclinic,  $a = 15.256(5)$ ,  $b = 7.788(3)$ ,  $c = 12.203(3)$  Å,  $\beta = 98.16(2)^\circ$ ,  $V = 1 435.2$  Å<sup>3</sup>,  $Z = 2$ ,  $D_c = 1.53$  g  $\text{cm}^{-3}$ ,  $F(000) = 692$ , space group  $P2_1$ , Mo- $K_\alpha$  X-radiation (graphite monochromator),  $\lambda = 0.7107$  Å.

**Structure Solution and Refinement.**—The structure was solved by direct methods using MULTAN<sup>13</sup> and in the final refinement by the block-matrix least-squares method; anisotropic thermal parameters were used for all non-hydrogen atoms except the atoms of the solvent. The contribution of hydrogen atoms was ignored. The refinement converged to  $R$  0.058 and  $R_w$  0.064. The data were not corrected for the effects of absorption. Positional parameters are shown in Table 7, and bond lengths and angles are summarized in Tables 3 and 4. A list of anisotropic thermal parameters are listed in Supplementary Publication No. SUP 56379 (2 pp.).\*

**Antifungal Test of the Metabolites.**—The antifungal activity was determined by the paper disc assay with *Gibberella zeae* (IFO 4474) and *Penicillium expansum* (IFO 5453) as test organisms. Fungi were cultivated in Czapek–Dox medium containing 1.6 w/v % agar. A 5-day-old mycelial suspension (1 ml) and the above medium (19 ml) were combined to prepare the assay plates. The metabolites, dissolved in acetone, methanol, or THF, were added to paper discs (8 mm diameter) to amounts of 100, 25, 10, 1, or 0.25 µg per disc, and placed on the assay plates. Zones of inhibition (mm in diameter) were recorded after 48 h incubation at 27 °C. The MIC was determined as described previously.<sup>14</sup>

**Table 7.** Final atomic fractional co-ordinates for non-hydrogen atoms with estimated standard deviations in parentheses

| Atom   | x            | y            | z           |
|--------|--------------|--------------|-------------|
| S(1)   | 0.187 5(2)   | 0.085 1(0)   | 0.917 5(3)  |
| S(2)   | 0.286 9(2)   | −0.094 2(4)  | 0.908 9(3)  |
| C(1)   | 0.383 7(6)   | 0.093 4(14)  | 1.062 9(8)  |
| C(3)   | 0.256 5(6)   | 0.260 5(13)  | 0.996 2(8)  |
| C(4)   | 0.310 3(6)   | 0.331 8(12)  | 0.906 6(8)  |
| C(5a)  | 0.413 8(6)   | 0.215 7(14)  | 0.775 2(8)  |
| C(6)   | 0.349 8(6)   | 0.211 4(14)  | 0.663 5(8)  |
| C(7)   | 0.400 2(7)   | 0.201 7(16)  | 0.565 3(9)  |
| C(8)   | 0.458 7(7)   | 0.091 1(17)  | 0.544 5(9)  |
| C(10)  | 0.486 1(8)   | −0.072 7(20) | 0.716 0(10) |
| C(10a) | 0.459 5(6)   | 0.042 5(14)  | 0.788 0(8)  |
| C(11)  | 0.465 4(7)   | −0.018 6(15) | 0.905 6(9)  |
| C(11a) | 0.382 0(6)   | 0.060 6(14)  | 0.940 2(8)  |
| C(12)  | 0.302 0(8)   | 0.208 3(18)  | 1.203 2(8)  |
| C(1')  | 0.108 5(6)   | 0.418 5(15)  | 0.953 5(8)  |
| C(2')  | 0.103 5(6)   | 0.546 2(13)  | 0.874 6(8)  |
| C(3')  | 0.027 9(6)   | 0.561 9(14)  | 0.796 7(8)  |
| C(4')  | −0.043 8(7)  | 0.453 5(14)  | 0.797 1(8)  |
| C(5')  | −0.042 4(7)  | 0.332 8(16)  | 0.883 2(10) |
| C(6')  | 0.034 0(7)   | 0.314 6(16)  | 0.962 1(9)  |
| C(7')  | 0.190 8(6)   | 0.389 2(14)  | 1.036 6(8)  |
| C(1'') | 0.099 2(6)   | 0.712 5(14)  | 0.666 8(8)  |
| C(2'') | 0.106 5(7)   | 0.873 3(14)  | 0.620 4(8)  |
| C(3'') | 0.182 0(6)   | 0.914 1(15)  | 0.572 1(9)  |
| C(4'') | 0.251 6(7)   | 0.797 0(13)  | 0.578 3(8)  |
| C(5'') | 0.241 3(6)   | 0.635 9(14)  | 0.624 5(8)  |
| C(6'') | 0.164 3(6)   | 0.591 0(14)  | 0.665 4(8)  |
| C(7'') | 0.317 0(6)   | 0.513 7(13)  | 0.636 0(8)  |
| C(8'') | 0.047 0(9)   | 1.153 3(16)  | 0.588 5(13) |
| N(2)   | 0.318 1(5)   | 0.192 6(12)  | 1.088 0(7)  |
| N(5)   | 0.366 2(5)   | 0.214 4(11)  | 0.874 7(6)  |
| O(1)   | 0.441 2(5)   | 0.028 3(11)  | 1.130 2(6)  |
| O(2)   | 0.299 3(4)   | 0.476 3(9)   | 0.869 1(5)  |
| O(3)   | 0.288 2(4)   | 0.351 2(8)   | 0.650 5(5)  |
| O(4)   | 0.541 1(5)   | 0.055 4(13)  | 0.966 8(7)  |
| O(9)   | 0.491 7(6)   | −0.049 9(11) | 0.606 0(7)  |
| O(1')  | 0.023 2(4)   | 0.681 5(10)  | 0.711 8(6)  |
| O(2')  | −0.114 8(5)  | 0.471 7(12)  | 0.717 6(6)  |
| O(3')  | 0.238 2(5)   | 0.542 9(10)  | 1.069 8(6)  |
| O(1'') | 0.037 6(5)   | 0.983 4(10)  | 0.624 5(7)  |
| O(2'') | 0.393 7(4)   | 0.551 5(10)  | 0.633 1(6)  |
| C(M)   | −0.338 3(9)  | 0.257 9(21)  | 0.700 6(12) |
| C(M')  | −0.172 9(10) | 0.897 3(26)  | 0.620 6(13) |
| O(M)   | −0.248 3(7)  | 0.267 5(16)  | 0.750 3(9)  |
| O(M')  | −0.128 7(7)  | 0.935 6(17)  | 0.723 2(9)  |

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## References

- 1 Part 7, K. Kawai, K. Nozawa, S. Nakajima, and Y. Iitaka, *Chem. Pharm. Bull.*, 1984, **32**, 94.
- 2 S. Udagawa and Y. Sugiyama, 'Reports on the Cryptogamic Study in Nepal, March 1982,' Miscellaneous Publication of the National Science Museum, Tokyo, 1982, p. 11.
- 3 H. Syea, S. Nakajima, K. Kawai, and S. Udagawa, *J. Chem. Soc., Chem. Commun.*, 1985, 657.

\* For details of the Supplementary Publication Scheme, see Instructions for Authors (1986), *J. Chem. Soc., Perkin Trans. 1*, 1986, Issue 1. Structure factors are available from the editorial office on request.

- 4 M. Yamazaki and Y. Maebayashi, *Chem. Pharm. Bull.*, 1982, **30**, 509.
- 5 R. Rahman, S. Safe, and A. Taylor, *J. Chromatogr.*, 1974, **53**, 592.
- 6 T. Kikuchi, S. Kadota, K. Nakamura, A. Nishi, T. Taga, T. Kaji, K. Osaki, and K. Tubaki, *Chem. Pharm. Bull.*, 1982, **30**, 3846.
- 7 R. Nagarajan and R. W. Woody, *J. Am. Chem. Soc.*, 1973, **95**, 7212.
- 8 R. Nagarajan, N. Neuss, and M. M. Marsh, *J. Am. Chem. Soc.*, 1968, **90**, 6518.
- 9 R. Nagarajan, L. L. Huckstep, D. H. Lively, D. C. DeLong, M. M. Marsh, and N. Neuss, *J. Am. Chem. Soc.*, 1968, **90**, 2980.
- 10 G. Diffieux, M.-A. Baute, R. Baute, and M.-J. Filleau, *J. Antibiot.*, (Tokyo), 1978, **31**, 1102.
- 11 D. Hauser, H. P. Weber, and H. P. Sigg, *Helv. Chim. Acta*, 1970, **53**, 1061.
- 12 Y. Maebayashi, Y. Horie, and M. Yamazaki, *Proc. Jpn. Assoc. Mycotoxicol.*, 1984, **20**, 28.
- 13 G. Germain, P. Main, and M. M. Woolfson, *Acta Crystallogr., Sect B*, 1970, **26**, 91.
- 14 K. Nozawa and S. Nakajima, *J. Nat. Prod.*, 1979, **42**, 374.

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