STUDIES ON FUNGAL PRODUCTS. XII.[1]
STRUCTURE OF AURANTIOEMESTRIN FROM EMERICELLA STRIATA

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Abstract ——— The structure of aurantioemestrin (**1**), a new degradated dioxopiperazine isolated from Emericella striata (80-NE-22), was established as **1** on the basis of spectroscopic evidences. Aurantioemestrin (**1**), a dioxopiperazinethione, seems to be the biogenetical key intermediate from emestrin (**3**) to dethiosecoemestrin (**2**).

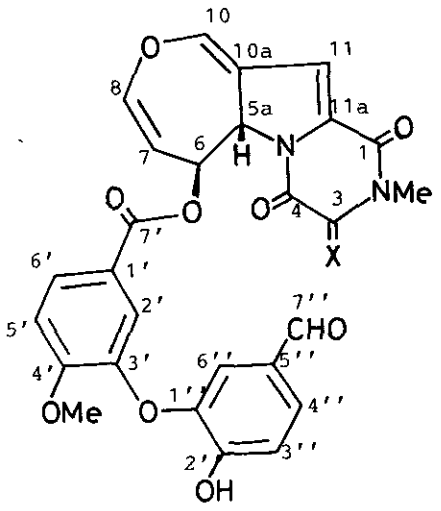
Previously we reported[2] the structural determination of dethiosecoemestrin (**2**), and the isolation of aurantioemestrin (**1**), both obtained from the methylene chloride extract of Emericella striata (Rai, Tewari & Mukerji) Malloch & Cain, strain 80-NE-22. The structural elucidation of **1**[3] is to be reported in this paper.

Aurantioemestrin (**1**), mp 118-120°C (dec.), $[\alpha]_D^{24}$ -556°, was isolated from the fraction slightly less polar than dethiosecoemestrin (**2**), of the methylene chloride extract of the culture filtrate. A slightly positive silver nitrate test (pale brown) suggested the presence of sulfur atom in **1**. The molecular formula of **1** was confirmed as $C_{27}H_{20}N_2O_9S$ from high resolution ms and elemental analysis. The ^1H and ^{13}C nmr spectra of **1** were closely similar to those of **2**, except the downfield shift of the protons assigned to N-methyl group at δ 3.292 in **2** to at δ 3.665 in **1**, and the carbon signal at δ 187.05 (Sq) in **1** instead of that at δ 157.45 (Sq). (Tables 1 and 2) The compound **1** was found to be easily degradated to dethiosecoemestrin (**2**). So aurantioemestrin (**1**)

Table 1. ^{1}H nmr chemical shifts of aurantioemestrin (1) and dethiosecoemestrin in (2) $CDCl_3$

Proton	1	2
2-NMe	3.665	3.292
5a-H	5.610	5.530
6-H	5.445	5.386
7-H	5.021	4.968
8-H	6.416	6.393
10-H	7.059	7.019
11-H	6.870	6.863
2'-H	7.425*	7.407
4'-OMe	3.915	3.899
5'-H	7.157	7.135
6'-H	7.584	7.571
3''-H	7.052	7.016
4''-H	7.988	7.942
6''-H	7.826*	7.744
7''-H	9.795	9.786

* The assignments may be reversed.



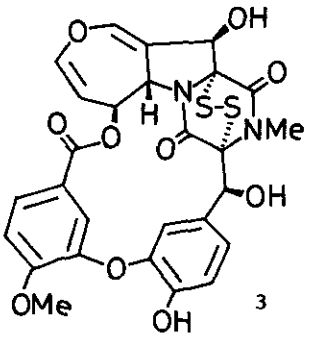
1 : X = S

2 : X = O

Table 2. ^{13}C nmr chemical shifts of aurantioemestrin (1) and dethiosecoemestrin (2) in $CDCl_3$

Carbon	1	2
C-1	154.07 (Sm)	155.86 (Sq)*
2-NMe	34.67 (Q)	27.31 (Q)
C-3	187.05 (Sq)	157.45 (Sq)*
C-4	148.15 (S)	149.16 (S)
C-5a	64.31 (Dm)	63.75 (Dm)
C-6	69.49 (Ddd)	69.41 (Ddd)
C-7	107.86 (Dm)	107.80 (Dm)
C-8	140.11 (Sm)	140.15 (Dm)
C-10	141.44 (Dm)	141.21 (Dbrd)
C-10a	119.28 (Sm)	118.88 (Sm)
C-11	122.21 (Dbrd)	122.22 (Dbrs)
C-11a	131.42 (Sd)	131.06 (Sd)
C-1'	122.31 (Sd)	122.18 (Sd)
C-2'	122.81 (Dd)	122.18 (Dd)
C-3'	143.96 (Sdd)	144.31 (Sdd)
C-4'	155.22 (Sm)	155.15 (Sm)
4'-OMe	56.15 (Q)	56.15 (Q)
C-5'	112.17 (D)	112.17 (D)
C-6'	128.70 (Dd)	128.57 (Dd)
C-7'	165.22 (Sbrdd)	165.22 (Sddd)
C-1''	145.37 (Sm)	145.05 (Sdd)
C-2''	153.07 (Sddd)	153.32 (Sddd)
C-3''	116.85 (D)	117.20 (D)
C-4''	127.61 (Dd)	127.57 (Dbrd)
C-5''	130.05 (Sdd)	130.12 (Sdd)
C-6''	118.14 (Dbrd)	118.72 (Dbrd)
C-7''	190.50 (Ddd)	190.53 (Ddd)

* The assignments may be reversed.



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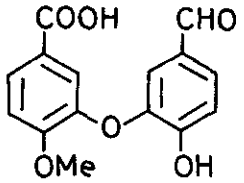

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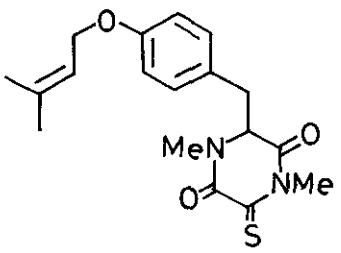
has a violaceic acid and a dihydrooxepine ring moieties in the molecule as same as **2**. Considering that compound **1** has a sulfur atom in the molecule instead of an oxygen atom in **2**, the structural difference between **1** and **2** should be at the trioxopiperazine (or dioxopiperazine) moiety. The carbon signal at δ 187.05 in **1** could be assigned as the carbon of a thioamide.[4] The coupling patterns of the above signal suggested that the thiocarbonyl located at C-3 position. The carbon signal at δ 187.02 in the ^{13}C nmr spectrum of silvathione (**5**), which was recently isolated from Aspergillus silvaticus Fennell & Raper, was also assigned to the same thioamide in a dioxopiperazinethione.[3] From all of these results, the structure of aurantioemestrin was confirmed as **1**.

The similarity of the coupling constants of the proton signals in the dihydrooxepine ring of **1** and **2** suggested that compound **1** has the same relative stereochemistry as **2**. The cd spectrum of **1** showed maxima at 282 (positive), 336 (negative), and 406 nm (negative), whereas that of **2** showed maxima at 250 (positive), 263 (positive), 304 (negative), and 362 nm (negative). These results suggested that **1** and **2** had the same absolute configurations around the dihydrooxepine ring, although about 20-30 nm of bathochromic shifts in the cd spectra of **1** compared with that of **2** were observed. Thus aurantioemestrin would have 5a*S*, 6*S* configuration, and consequently the absolute structure of the same compound should be as depicted in **1**.

Many epidithiodioxopiperazine derivatives have been isolated from fungi, but there are only a few examples of the concurrent isolation of the trioxopiperazines from the same fungus; dethiosecoemestrin (**2**)[2] accompanied with emestrin (**3**) in E. striata, dioxopiperazinoindoles (**6** and **7**) along with dehydrogliotoxin (**8**) in Penicillium terlikowskii Zaleski (Syn. P. spinulosum Thom).[5,6] Our present isolation of aurantioemestrin (**1**) is the first case of the isolation of the dioxopiperazinethione along with the epidithiodioxopiperazine, emestrin (**3**), and the trioxopiperazine, dethiosecoemestrin (**4**) from the same fungus E. striata. Chemically compound **1** is easily degradated to **2**, which is further degradated slowly to **4**. And it seems to be clear that the dioxopiperazinethiones are the important key intermediates from epidithiodioxopiperazines to the biosynthetically accompanied trioxopiperazines. The structures of silvathione and a corresponding epidithiodioxopiperazine from A. silvaticus will be reported in another paper.



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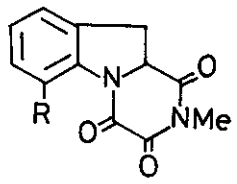
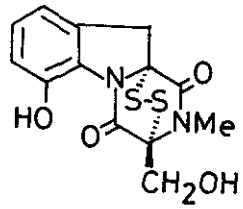


6 : R = H

7 : R = OH



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EXPERIMENTAL

Melting points were uncorrected. The following instruments were used: optical rotation; JASCO DIP-181 spectrometer: mass spectra; JEOL JMS-D 300 spectrometer: uv spectra; Hitachi 124 spectrometer: ir spectra; Hitachi 215 spectrometer: ^{1}H and ^{13}C nmr spectra; JEOL JNM-GX 400 spectrometer: cd curves; JASCO J-40 spectrometer. *Abbreviations*: S or s = singlet, D or d =doublet, Q or q = quartet, br = broad, sh = shoulder.

Isolation of Aurantioemestrin (1)

The methylene chloride extract (7.2 g) of the acidified culture filtrate (50 l) of Emericella striata (80-NE-22) was chromatographed on silica gel with benzene-acetone (20:1) and chloroform-methanol (200:1) successively, followed by low pressure liquid chromatography (glass column packed with silica gel) using the solvent system of benzene-acetone (20:1) to give pure aurantioemestrin (1, 46 mg).

Aurantioemestrin (1)

Compound 1 was obtained as orange crystalline powder, mp 118–120°C, $[\alpha]_D^{24}$ -556° (c=0.412 in chloroform). ir ν_{max}^{KBr} cm^{-1}: 3350 (OH), 1710 (COO), 1690, 1670 (CON), 1595. uv λ_{max}^{EtOH} nm (log ε): 227 sh (4.53), 263 (4.45), 287 sh (4.36), 340 (4.25), 418 (4.01). field desorption ms m/z: 548 (M^+, 100%), 288 (13%). high resolution ms m/z: 548.0864 (M^+, 548.0887 for $C_{27}H_{20}N_2O_9S$). Anal. Calcd for $C_{27}H_{20}N_2O_9S$: C, 59.12; H, 3.67; N, 5.11. Found: C, 59.45; H, 3.87; N, 4.99. ^{1}H nmr ($CDCl_3$) δ: 3.665 (3H, s, NMe), 3.915 (3H, s, OMe), 5.021 (1H, brd, J=8.3 Hz, 7-H), 5.445 (1H, ddd, J=8.3, 2.0 and 2.0 Hz, 6-H), 5.610 (1H, dd, J=8.3 and 2.0 Hz, 5a-H), 6.416 (1H, dd, J=8.3 and 2.0 Hz, 8-H), 6.870 (1H, s, 11-H), 7.052 (1H, d, J=8.4 Hz, 3''-H), 7.059 (1H, brs, 10-H), 7.157 (1H, d, J=8.2 Hz, 5'-H), 7.425 (1H, d, J=1.8 Hz, 2'-H), 7.584 (1H, dd, J=8.2 and 1.8 Hz, 6'-H), 7.826 (1H, d, J=1.8 Hz, 6''-H), 7.988 (1H, dd, J=8.4 and 1.8 Hz, 4''-H), 9.795 (1H, s, CHO). ^{1}H

nmr (C_6D_6) δ: 3.126 (6H, s, NMe and OMe), 4.661 (1H, dd, J=8.2 and 1.7 Hz, 7-H), 5.211 (1H, dd, J=8.2 and 2.5 Hz, 5a-H), 5.346 (1H, ddd, J=8.2, 2.4 and 1.7 Hz, 6-H), 5.837 (1H, dd, J=8.2 and 2.4 Hz, 8-H), 6.084 (1H, s, 11-H), 6.160 (1H, d, J=2.5 Hz, 10-H), 6.478 (1H, d, J=8.5 Hz), 7.050 (1H, d, J=8.2 Hz), 7.363 (1H, dd, J=8.2 and 1.7 Hz), 7.676 (1H, d, J=1.7 Hz), 8.144 (1H, dd, J=8.5 and 1.9 Hz), 8.217 (1H, d, J=1.9 Hz), 9.640 (1H, s, CHO). cd (c=9.4×10^{-4} in methanol) $[\theta]_{282}$ $+5.04\times10^4$, $[\theta]_{336}$ -4.03×10^4, $[\theta]_{406}$ -1.43×10^4. ^{13}C Nmr signals in $CDCl_3$ are listed in Table 2.

Degradation of aurantioemestrin (1)

Compound **1** was stood in ethanol solution for 4 days to give dethiosecoemestrin (**2**), which was identified with uv spectrum and thin layer chromatographic (tlc) and high performance liquid chromatographic (hplc) behaviours, and violaceic acid (**4**), which was identified by tlc and hplc behaviours.

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