

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

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Abstract — The structure of aurantioemestrin (1), a new degraded 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).

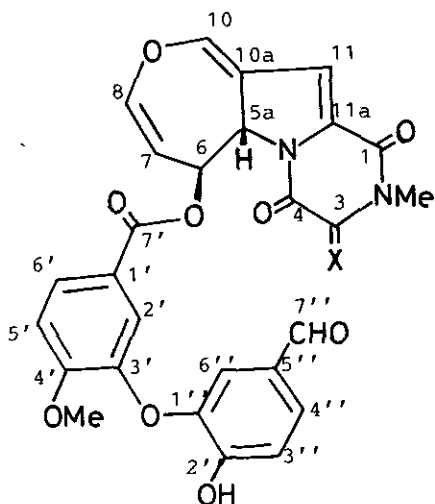
Previously we reported² the structural determination of dethiosecoemestrin (2), and the isolation of aurantioemestrin (1), both obtained from the methylene chloride extract of Emericella striata (Rai, Tewari & Mukerji) Malloch & Cain, strain 80-NE-22. The structural elucidation of 1³ is to be reported 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 C₂₇H₂₀N₂O₉S from high resolution ms and elemental analysis. The ¹H and ¹³C 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 degraded to dethiosecoemestrin (2). So aurantioemestrin (1)

Table 1. ^1H 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.

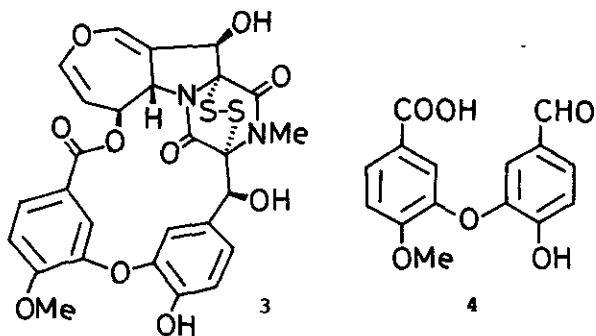


1 : X = S
2 : X = O

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)

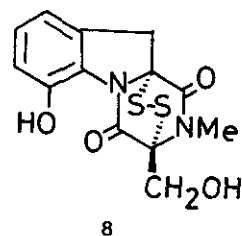
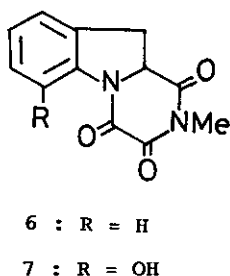
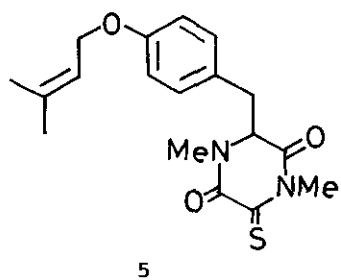
* The assignments may be reversed.



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 ¹³C 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 \underline{S} , 6 \underline{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 degraded to 2, which is further degraded 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.



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: ^1H 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, $[\alpha]_D^{24} -556^\circ$ (c=0.412 in chloroform). ir $\nu_{\text{max}}^{\text{KBr}} \text{ cm}^{-1}$: 3350 (OH), 1710 (COO), 1690, 1670 (CON), 1595. uv $\lambda_{\text{max}}^{\text{EtOH}}$ 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 $\text{C}_{27}\text{H}_{20}\text{N}_2\text{O}_9\text{S}$). Anal. Calcd for $\text{C}_{27}\text{H}_{20}\text{N}_2\text{O}_9\text{S}$: C, 59.12; H, 3.67; N, 5.11. Found: C, 59.45; H, 3.87; N, 4.99. ^1H 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). ^1H

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 ($c=9.4 \times 10^{-4}$ in methanol) $[\theta]_{282} +5.04 \times 10^4$, $[\theta]_{336} -4.03 \times 10^4$, $[\theta]_{406} -1.43 \times 10^4$. ^{13}C Nmr signals in $CDCl_3$ are listed in Table 2.

Degradation of auranioemestrin (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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