Pergamon

PII: S0031-9422(97)00210-0

Phytochemistry, Vol. 46, No. 1, pp. 123–126, 1997

0031-9422/97 $17.00+0.00

AN EPITETRATHIODIOXOPIPERAZINE RELATED TO EMESTRIN FROM *EMERICELLA FOVEOLATA*

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(*Received* 18 *December* 1996)

Key Word Index—*Emericella foveolata*; *E. striata*; fungi; epipolythiodioxopiperazine; secoemestrin C; emestrin; emestrin B; aurantioemestrin; dethiosecoemestrin.

Abstract—An epitetrathiodioxopiperazine, secoemestrin C, related to the macrocyclic antifungal epidithiodioxopiperazine, emestrin, originally isolated from *Emericella striata* and *E. quadrilineata*, was isolated along with the epitrithiodioxopiperazine, emestrin B, from the culture medium of the early state of *E. foveolata*. This fungus also produced emestrin in the mycelium and dethiosecoemestrin and violaceic acid in the culture broth after two weeks cultivation. The structure of secoemestrin C was established by spectroscopic and chemical investigation. Secoemestrin C may be the key intermediate from emestrin and/or emestrin B to dethiosecoemestrin.

INTRODUCTION

The novel antifungal macrocylic epipolythiodioxopiperazines, emestrin (**1**) [1] and emestrin B (**2**) [2], have been isolated from mycelial extracts of *Emericella striata*. Emestrin (**1**) was also isolated from *E. quadrilineata*, as mycotoxin EQ-1 [3], *E. nidulans* var. *acristata* and *E. parvathecia*. Positive coloration (dark brown) with aqueous silver nitrate [4] was used for detecting epipolythiodioxopiperazines on TLC. From the extract of the culture filtrate of *E. striata*, *E. quadrilineata* and *E. nidulans* var. *acristata*, dethiosecoemestrin (**3**) [5], aurantioemestrin (**4**) [6] and violaceic acid (**5**) [5], which was originally isolated from *E. violacea* [7], were isolated. It was confirmed that **4** was degradated to **3** and finally to **5** [6]. In the course of a search for a key intermediate for **1** to **4**, the new compound (**6**) positive to silver nitrate reagent was detected on TLC along with **3** and **5** from culture filtrates of *E. foveolata*, whereas **1** was isolated from the mycelium. The amount of **6** increased during the first week and then decreased slowly. This compound, designated secoemestrin C, was isolated along with **5** and **2** from the culture filtrate of the early state (6 days) on cultivating this fungus. The paper deals mainly the structural elucidation of **6**.

RESULTS AND DISCUSSION

The $[M]^+$ of secoemestrin C (**6**) was not detected on EI mass spectrometry, but its molecular formula was confirmed as $C_{27}H_{22}N_2O_{10}S_4$ by high resolution positive FAB mass spectrometry at *m/z* 663 $[M+H]^+$ and negative FAB mass spectrometry at *m/z* 661 $[M-H]^-$. The highest peak at *m/z* 534, which corresponded with $C_{27}H_{22}N_2O_{10}$, in the EI mass spectrum of **6**, was assigned as the partial structure lacking four sulphur atoms.

The ^1H NMR signals at δ 8.14, 8.07 and 7.08, and 7.60, 7.56, and 7.13 for **6** in $CDCl_3$ (Table 1) confirmed the presence of two 1,2,4-trisubstituted benzene moieties. In addition, signals for one methoxyl (δ 3.94) and one aldehyde (δ 9.78) group were observed. These signals and the strong peak at *m/z* 288 ($C_{15}H_{12}O_6$) in the EI mass spectrum showed the presence of a violaceic acid (**5**) moiety in **6**, as in dethisecoemestrin (**3**) [5] and aurantioemestrin (**4**) [6]. Six ^1H NMR signals at δ 6.86, 6.32, 5.48, 5.33, 4.90 and 4.89 were similar to those observed in the dihydrooxepine and neighbouring five-membered rings of emestrin (**1**) and emestrin B (**2**). These coupling patterns were confirmed as the same as in **1** and **2** by $^1H-^1H$ decoupling experiments. The remaining ^1H NMR signals were observed at δ 3.04 (3H) and 5.24 (1H). The former was assigned to an *N*-methyl group, whereas the latter should be the proton attached to the carbon bearing the carbonyl carbon and the amide nitrogen, as in dithiosivatin (**7**) (δ 5.33) [8], which was isolated from *Aspergillus silvaticus*. The above results suggested that the structure of secoemestrin C is an epitetrathiodioxopiperazine derivative as depicted in **6**.

Since **6** was slowly degraded in $CDCl_3$ solution, the ^{13}C NMR, ^1H-detected heteronuclear multiple-quantum coherence via direct coupling (HMQC), and

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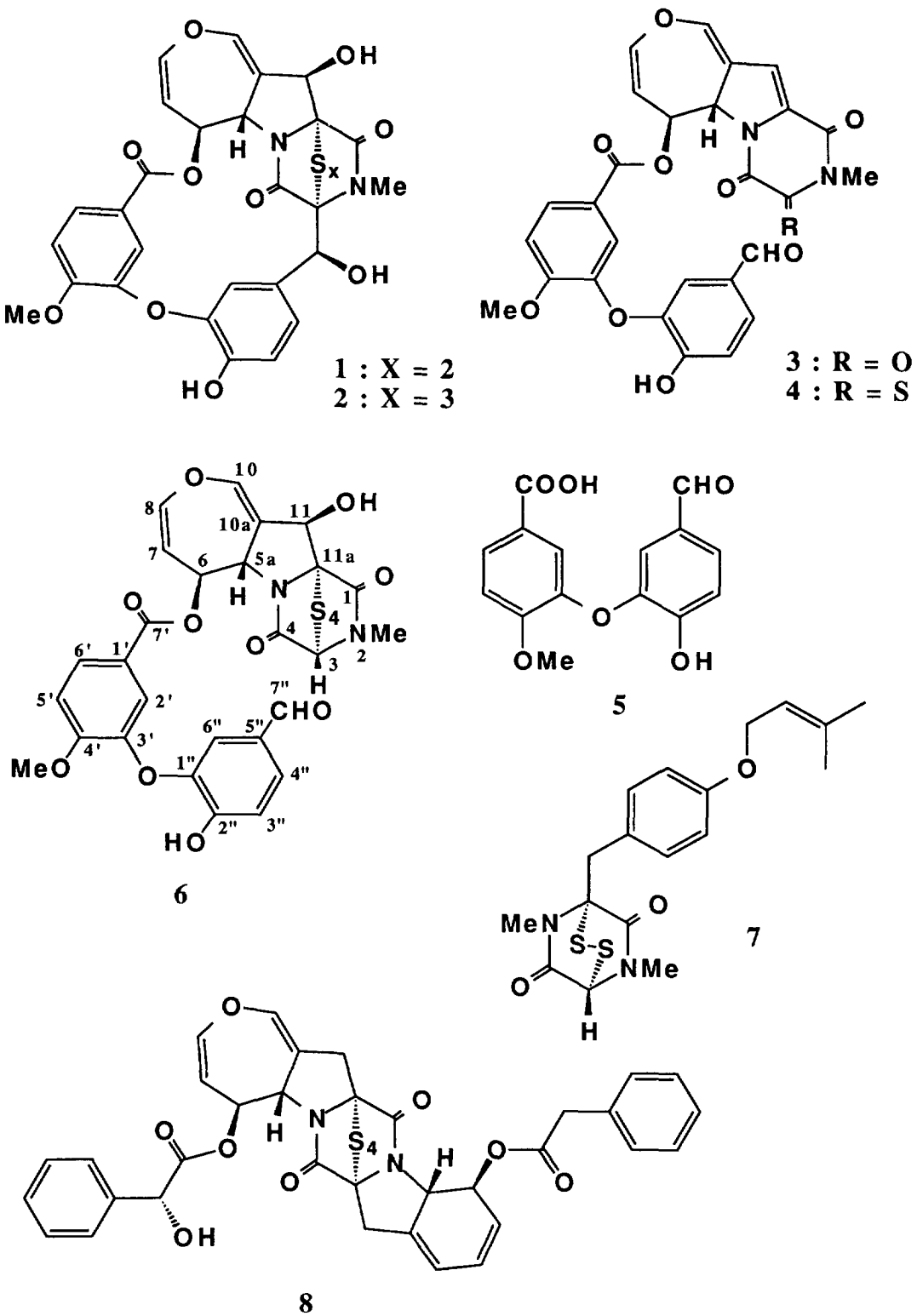

heteronuclear multiple bond connectivity by 2D multiple quantum NMR (HMB C) spectra were taken using Me_2CO-d_6 as solvent. The relative structure of secoemestrin C (**6**) was confirmed by means of HMBC correlations (Fig. 1) and decoupling experiments. Comparison of the CD curve of **6** [230 (negative) and 326 (negative) nm] with those of emestrin (**1**) [233 (negative), 266 (positive), and 338 (negative) nm] and emethallicin B (**8**) [236 (negative), 272 (positive) and 300 (negative) nm] [9], isolated from *E. hetero-thallica*,

Table 1. ^1H and ^{13}C NMR chemical shifts of secoemestrin C (**6**) and related compounds in $CDCl_3$

Carbon no.	**6**[a] δ_C	δ_H	**6** δ_H	**1**[b] δ_C	δ_H	**3** δ_C	δ_H	**4** δ_C	δ_H
1	166.7			164.3		155.9		154.1	
2-NMe	32.3	2.98	3.04	27.3	3.26	27.3	3.29	34.7	3.67
3	68.6	5.43	5.24	75.6		157.5		187.1	
4	164.3			160.6		149.2		148.2	
5a	60.2	5.35	5.48	60.0	5.67	63.8	5.53	64.3	5.61
6	72.7	5.28	5.33	72.9	4.67	69.4	5.39	69.5	5.45
7	108.5	4.93	4.89	107.2	4.91	107.8	4.97	107.9	5.02
8	140.0	6.43	6.32	137.2	6.41	140.2	6.39	140.1	6.42
10	142.3	7.03	6.86	141.7	7.06	141.2	7.02	141.4	7.06
10a	113.2			112.4		118.9		119.3	
11	78.6	4.95	4.90	74.8	5.47	122.2	6.86	122.2	6.87
11-OH		5.38	—		6.25				
11a	81.1			81.0		131.1		131.4	
1′	123.8			122.2		122.2		122.3	
2′	122.9	7.82	8.14	124.0	7.38	122.2	7.74[d]	122.8	7.83[d]
3′	145.4			143.4[c]		144.3		144.0	
4′	156.2			148.9		155.2		155.2	
4′-OMe	56.7	3.93	3.94	55.8	3.94	56.2	3.90	56.2	3.92
5′	113.2	7.27	7.08	115.4	7.21	112.2	7.02[e]	112.2	7.05[e]
6′	129.0	8.03	8.07	124.9	7.58	128.6	7.94[f]	128.7	7.99[f]
7′	165.8			164.4		165.2		165.2	
1″	146.5			145.3[c]		145.1		145.4	
2″	154.6			153.3		153.3		153.1	
2″-OH		9.18	—		9.74		10.73		
3″	117.7	7.17	7.13	112.3	6.88	117.2	7.14[e]	116.9	7.16[e]
4″	128.5	7.61	7.56	122.7	7.17	127.6	7.57[f]	127.6	7.58[f]
5″	130.8			127.1		130.1		130.1	
6″	118.5	7.34	7.60	120.4	7.77	118.7	7.41[d]	118.1	7.43[d]
7″	190.9	9.78	9.78	74.8	4.97	190.5	9.79	190.5	9.80
7″-OH					5.99				

[a] Measured in $(CD_3)_2CO$.
[b] Measured in $(CD_3)_2SO$.
[c] Assignments may be reversed.
[d,e,f] Assignments reversed.

indicated that these three compounds had the same configuration about the epipolythiodioxo-piperazine ring [10]. The positive Cotton effect at *ca* 270 nm in **6** would disappear because of the large negative Cotton effect at 263 nm (attributed to the benzene moiety). Thus, the structure of secoemestrin C, including the absolute stereochemistry, was confirmed as **6**.

Secoemestrin C is the first example of an epitetrathiodioxopiperazine derivative isolated from an emestrin (**1**)-producing fungus. The dioxopiperazinethione derivatives, aurantioemestrin (**4**) and silvathione [8], have been isolated previously from *E. striata* and *A. silvaticus*, respectively. We mentioned that these compounds might be the intermediate from epipolythiodioxopiperazine derivatives, like **1**, to trioxopiperazines, like dethiosecoemestrin (**3**) [6]. Degradation from **4** to **5** via **3** was recognized in ethanol solutions of **4**. It is noteworthy that **6** was isolated not from the mycelium but from the culture broth, along with emestrin B (**2**); the amount of **6** was maximal after 6 days cultivation of *E. foveolata*. Thus, secoemestrin C (**6**) may be the key intermediate from emestrin (**1**) and/or emestrin B (**2**) to aurantioemestrin (**4**).

EXPERIMENTAL

General. Mps: uncorr. FAB MS: *m*-nitrobenzyl alcohol as matrix. ^1H (500 MHz) and ^{13}C (125 MHz) NMR: TMS as int. standard. LPLC: glass column (300 × 10 i.d. mm) with silica gel CQ-3 (Wako). HPLC: YMC-Pack SIL-06 (300 × 10 i.d. mm). TLC: Kieselgel 60 F_{254} plates (Merck); detection by spraying 5% $AgNO_3$ soln and then heating.

Isolation of secoemestrin C (**6**). *Emericella foveolata* Horie, strain IFO 30559 (=IFM4547), was cultivated on Czapek medium (30 l) supplemented with 0.2% yeast extract using 180 Roux flasks at 27° for 6 days. After removal of mycelia by filtration, the culture filtrate was extracted with CH_2Cl_2 at pH 3 and the organic layer was dried (Na_2SO_4) and then evapd *in vacuo*. The obtained extract (1.02 g) was chromatographed on silica gel with $CHCl_3$–Me_2CO (6:1), followed by repeated purification by LPLC with

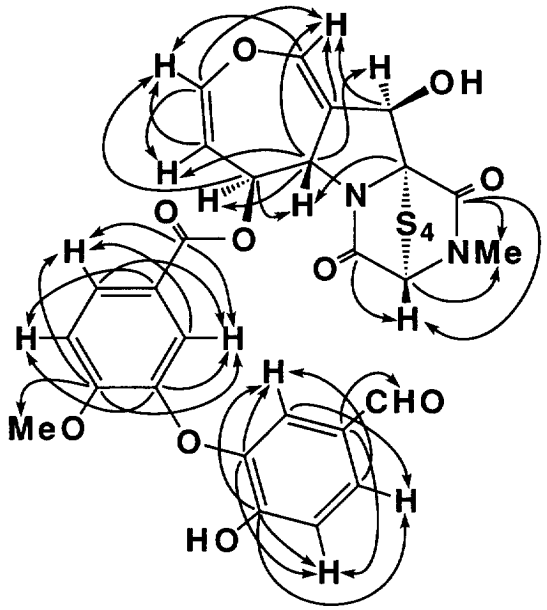

Fig. 1. Correlations in HMBC spectrum os secoemestrin C (**6**) Arrows indicate correlations from carbon (C_A) to proton (H_B): $C_A \rightarrow H_B$.

$CHCl_3$–Me_2CO (6:1) and/or cyclohexane–Me_2CO (3:1), to obtain secoemestrin C (**6**) (60 mg) and emestrin B (2 mg).

Secoemestrin (**6**). Amorphous, mp 164–166°. $[\alpha]_D^{20}$ −108.5° (Me_2CO, *c* 0.8). $AgNO_3$ test: positive (dark brown). EIMS (probe), *m/z* (rel. int): 534.1275 $[M-S_4]^+$ (534.1275 for $C_{27}H_{22}N_2O_{10}$, 1.1), 505 (0.5), 288.0634 $[C_{15}H_{12}O_6]^+$ (288.0634 for $C_{15}H_{12}O_6$, 48). Positive FAB MS *m/z* (rel. int.): 685 $[M+Na]^+$ (0.1), 663.0240 $[M+H]^+$ (663.0233 for $C_{27}H_{23}N_2O_{10}S_4$, 1.3), 534 $[M-S_4]^+$ (4), 505 (2), 375 (4). Negative FAB-MS *m/z* (rel. int.): 815 $[M+NBA]^-$ (0.9), 661 $[M-H]^-$ (6), 597 $[M-S_2-H]^-$ (16), 565 $[M-S_3-H]^-$ (12), 533 $[M-S_4-H]^-$ (2). UV λ_{max}^{MeOH} nm (log ε): 222 (4.79), 265 (4.35), 289 *sh* (4.21), 344 (3.27). IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 1715 (-COO-), 1700 (-CO-), 1680, 1650 (-CON-). CD (*c* 5.1×10^{-5}, MeOH) $\Delta\varepsilon^{20}$ (nm): −38.2 (230), −24.0 (248), +1.7 (303), −3.2 (326) ^1H NMR: Table 1. ^{13}C NMR: Table 1.

Acknowledgements—We thank Dr M. Kubo of Tsumura and Co. for FAB mass measurements. We are also grateful to Mrs T. Ogata, Mrs Y. Kawada, and Mr H. Komiya of Hoshi University for elemental analysis and NMR and mass measurements. This work was supported in part by a Grant-in-Aid for Scientific Research (no. 07672296) from the Ministry of Education Science, Sports and Culture, Japan.

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