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Studies on Fungal Products. IX.[1] Dethiosecoemestrin, a New Metabolite Related to Emestrin, from *Emericella striata*

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In the course of searching for metabolites related to emestrin (**3**) from *Emericella striata* (80-NE-22), dethiosecoemestrin (**1**) was isolated from the methylene chloride extract of the culture filtrate. The tremorgenic mycotoxin, paxilline (**6**), was also isolated from the mycelial extract. The structure of dethiosecoemestrin was established on the basis of the chemical and spectroscopic evidence as **1**. It is postulated that dethiosecoemestrin was biogenetically derived from emestrin. Dethiosecoemestrin was easily degradated to violaceic acid (**5**). The antimicrobial activity of dethiosecoemestrin was examined.

Keywords——*Emericella striata*; mycotoxin; dioxopiperazine; trioxopiperazine; dethiosecoemestrin; emestrin; paxilline; violaceic acid

In the previous paper we reported[1,2] the isolation and structural elucidation of the antifungal epidithiodioxopiperazine, emestrin (**3**), and a related compound, violaceic acid (**5**), and the presence of sterigmatocystin, which is a representative mycotoxin of *Aspergillus nidulans* group (teleomorph: *Emericella*). In the course of searching for metabolites other than **3** or **5** from *E. striata* (RAI, TEWARI *et* MUKERJI) MALLOCH *et* CAIN (80-NE-22), three compounds were isolated: two new compounds designated as dethiosecoemestrin (**1**) and aurantioemestrin, and paxilline (**6**), a tremorgenic metabolite.[3] The structural elucidation of **1** is to be reported in this paper.

Dethiosecoemestrin (**1**), mp 136 °C (dec.), and aurantioemestrin, mp 118—120 °C (dec.), were isolated from the less polar fraction than **5** obtained from the methylene chloride extract of the culture filtrate. The molecular formula of **1** was confirmed as $C_{27}H_{20}N_2O_{10}$ from the field desorption (FD) mass spectrum (MS) and the elemental analysis. The proton nuclear magnetic resonance (^{1}H-NMR) signals at δ 7.016, 7.135, 7.407, 7.571, 7.744, and 7.942 of **1** (Fig. 1) confirmed the presence of two 1,2,4-trisubstituted benzene moieties. Five ^{1}H-NMR signals at δ 4.968, 5.386, 5.530, 6.393, and 7.019, and six carbon-13 nuclear magnetic resonance (^{13}C-NMR) signals at δ 63.75 (Dm), 69.41 (Ddd), 107.80 (Dm), 118.88 (Sm), 140.15 (Dm), and 141.21 (Dbr d) are similar to those observed in the dihydrooxepine moiety of **3** and its derivative (**4**). Homonuclear ^{1}H-{^{1}H} decoupling experiments (Fig. 1) also confirmed the presence of the dihydrooxepine moiety in the molecule of **1**. On acetylation, **1** afforded a monoacetate (**2**), mp 140—142 °C, $C_{29}H_{22}N_2O_{11}$, which showed ^{1}H-NMR signals at δ 2.221, assigned to the methyl protons of an aromatic acetoxyl group. No absorption of a hydroxyl group was observed in the infrared (IR) spectrum of **2**. Thus the ^{1}H-NMR signal at δ 6.863 in **1** can be assigned to an olefinic proton. This proton correspons to one of two olefinic protons at δ 6.52 (s) and 6.67 (s) in **4**. Bathochromic shifts in the ultraviolet (UV) spectra of **1** (365 nm) and **2** (367 nm) compared with that of **3** (278 nm) were observed, corresponding well to the

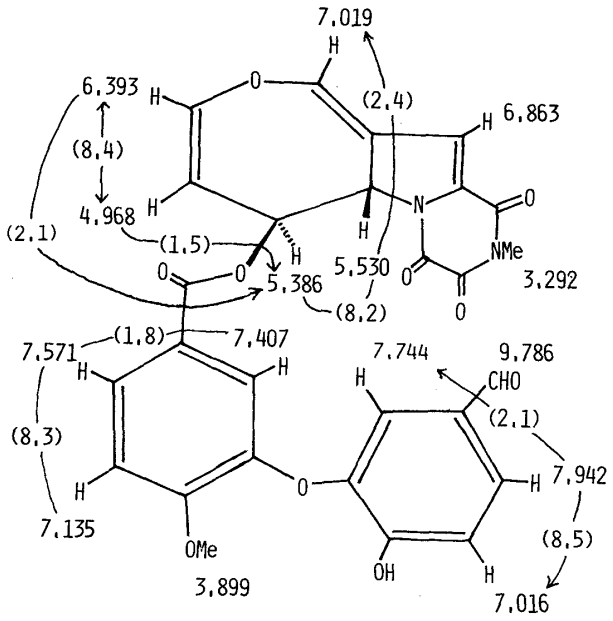


Fig. 1. ^{1}H-NMR Chemical Shift Assignments of Dethiosecoemestrin (**1**)

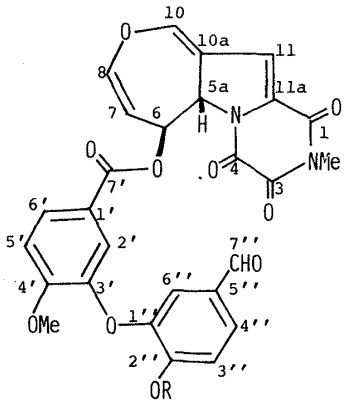
Arrow ($H_a \rightarrow H_b$) indicates that the proton b was decoupled when the proton a was irradiated. The coupling constants are given in parentheses.

TABLE I. ^{13}C-NMR Chemical Shifts of Dethiosecoemestrin (**1**) and Related Compounds in $CDCl_3$

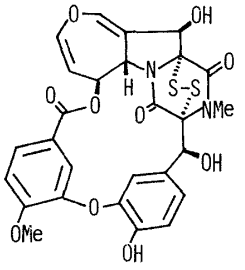
Carbon No.[a]	**1**	**4**	**5**[b]
1	155.86 (Sq)[c]	156.67 (S)[c]	—
2-NMe	27.31 (Q)	29.92 (Q)	—
3	157.45 (Sq)[c]	134.61 (S)	—
4	149.16 (S)	154.56 (S)[c]	—
5a	63.75 (Dm)	63.40 (D)	—
6	69.41 (Ddd)	71.18 (D)	—
7	107.80 (Dm)	107.89 (D)	—
8	140.15 (Dm)	138.14 (D)[d]	—
10	141.21 (Dbr d)	139.59 (D)[d]	—
10a	118.88 (Sm)	119.24 (S)	—
11	122.22 (Dbr s)	119.17 (D)[e]	—
11a	131.06 (Sd)	132.99 (S)	—
1′	122.18 (Sd)	121.03 (S)	123.2 (Sd)
2′	122.18 (Dd)	123.21 (D)[e]	118.7 (Dd)
3′	144.31 (Sdd)	140.58 (S)	144.3 (Sm)
4′	155.15 (Sm)	146.11 (S)	154.3 (Sdd)
4′-OMe	56.15 (Q)	55.84 (Q)	55.9 (Q)
5′	112.17 (D)	112.40 (D)	112.4 (D)
6′	128.57 (Dd)	126.32 (D)	126.2 (Dd)
7′	165.22 (Sddd)	165.58 (S)	166.4 (Sdd)
1″	145.05 (Sdd)	144.41 (S)	144.6 (Sm)
2″	153.32 (Sddd)	154.11 (S)	153.7 (Sm)
3″	117.20 (D)	119.48 (D)[e]	117.0 (D)
4″	127.57 (Dbr d)	121.60 (D)[e]	127.8 (Dbr d)
5″	130.12 (Sdd)	132.81 (S)	128.8 (Sdd)
6″	118.72 (Dbr d)	120.96 (D)[e]	118.7 (Dd)
7″	190.53 (Ddd)	116.91 (D)	190.5 (Ddd)

a) Numberings of the related compounds correspond to that of **1**. *b*) This compound was measured in DMSO-d_6. *c*—*e*) The assignments may be reversed.

UV absorption at 360 nm in **4**. The ^{13}C-NMR signals of **1** were more similar to those of **4** than **3**. Only two carbons, *i.e.*, those at δ 134.61 (S) and 116.91 (D) in **4**, were greatly changed to δ 157.45 (Sq) and 190.53 (Ddd) in **1**, respectively, as shown in Table I. The above results suggested that dethiosecoemestrin (**1**) is closely similar in structure to *O*-acetyldianhydrodidethiosecoemestrin (**4**), except for the functional groups at C-3 and C-7″.



1 : R = H
2 : R = Ac



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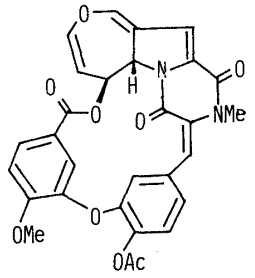

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Chart 1

The ^{13}C-NMR signal at δ 190.53 in **1** was assigned to an aldehyde, and the proton of this aldehyde appeared at δ 9.786. The ^{1}H-NMR signals of **1** and the comparison of the ^{13}C-NMR spectra of **1** and **5** (Table I) suggested that **1** had a violaceic acid moiety as a partial structure. The fragment ions at *m/z* 288 (base peak), 239, 152, and 135 in the electron impact (EI) MS of **1** also corresponded to those of **5** in all respects. Compound **1** was slowly degradated in an organic solvent such as methanol to give violaceic acid (**5**). The absorption at 1715 cm^{-1} in the IR spectrum of **1** suggested the presence of an ester. These results confirmed that dethiosecoemestrin (**1**) contains a violaceic acid moiety linked at the C-6 position as the ester.

The absorptions at 1680 and 1670 cm^{-1} in the IR spectrum of **1** suggested the presence of amides. Dethiosecoemestrin (**1**) was hydrogenated with platinum in order to obtain the hydrogenated derivative for the determination of the partial structure around the amides, but only violaceic acid (**5**) was obtained. Three upfield carbonyl signals at δ 157.45 (Sq), 155.86 (Sq), and 149.16 (S) in **1** corresponded with three amide carbons at δ 160.2, 157.0, and 152.0 in neoechinulin (**7**) isolated from *Aspergillus amstelodami* (teleomorph: *Eurotium*)[4] and with three carbons at δ 166.3, 157.0, and 152.5 in **8**.[5] Therefore those three signals of **1** can be assigned to amide carbonyls of the trioxopiperazine moiety. The mass spectral ion at *m/z* 244 in **1** is considered to be the ion shown as **9** in Chart 2. From all these results, the structure of dethiosecoemestrin was determined as **1**.

The circular dichroism (CD) spectrum of **1** showed maxima at 250 (positive), 263 (positive), 304 (negative), and 362 nm (negative), whereas that of **4** showed maxima at 249 (positive), 266 (positive), 297 (negative), and 358 nm (negative). Thus it is clear that **1** and **4** have the same configurations around the dihydrooxepine ring. Dethiosecoemestrin must therefore have 5a*S*, 6*S* configurations and consequently the absolute structure of dethiosecoemestrin is as depicted in **1**.

Emestrin was first isolated from *Emericella quadrilineata* by Maebayashi *et al.*[6] as a mycotoxin. Paxilline is a tremorgenic prenylated indole first isolated from *Penicillium paxilli* by Cole *et al.*[7] This is the first record of the isolation of this compound from a member of the genus Aspergillus. At a 25 mg/kg dose of **6**, tremors were caused in cockerels, and some

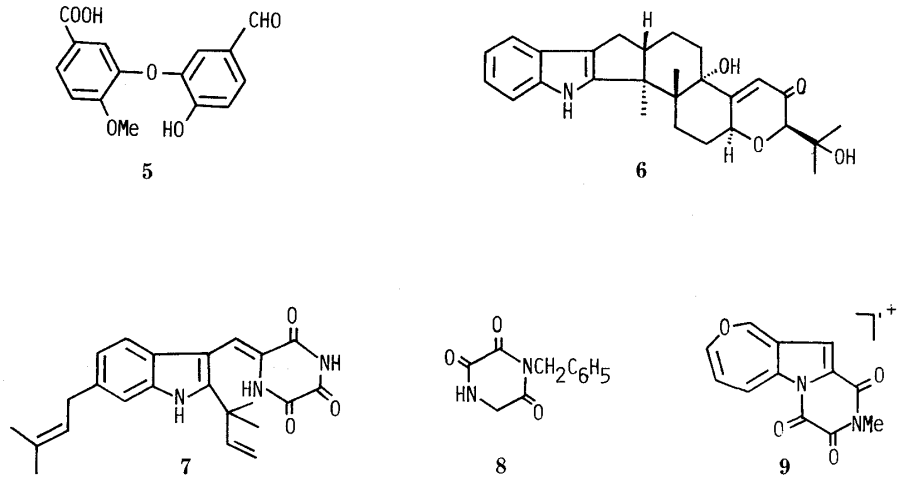

Chart 2

TABLE II. Antibacterial Activities of Metabolites Isolated from *Emericella striata*

Compound	Test organisms and amount of metabolites (μg/disc) *E. coli*					*B. subtilis*				
	100	25	10	2.5	1	100	25	10	2.5	1
1	−[a]	−	−	−	−	16[b]	15	15	±[c]	−
3	14	13	11	±	±	15	14	13	12	±
5	±	−	−	−	−	−	−	−	−	−
9	−	−	−	−	−	−	−	−	−	−

a) Minus (−) means no inhibition. *b*) Diameter of inhibition circle (mm). *c*) Plus-minus (±) means that clear inhibition was not detectable unequivocally.

cockerels died at 100—500 mg/kg.[7] Thus the mycotoxins associated with *E. striata* are sterigmatocystin,[1] emestrin (**3**), and paxilline (**6**). Among them, only sterigmatocystin has received much attention from mycotoxicologists, since it was discovered to occur in nature in a variety of products such as cheese, stored wheat, barley and rice, green coffee beans, corn, and commercial mixed feed. Emestrin and paxilline have not yet been detected as natural food contaminants nor have they been directly implicated in any animal disease outbreak. However, it is interesting that three different types of mycotoxins were isolated from the single fungus, *E. striata*.

In the previous paper,[1] we reported that only emestrin (**3**) among the metabolites of *E. striata* had antifungal activity. The antibacterial activities of the major metabolites are summarized in Table II. Emestrin (**3**) inhibited the growth of *Escherichia coli* and *Bacillus subtilis* at concentrations of 10 and 2.5 μg/disc, respectively, and the activities seemed to be slightly weaker than those against fungi. Dethiosecoemestrin (**1**) was also found to possess antibacterial activity at 10 μg/disc against *E. coli*, a gram-negative bacterium. Paxilline (**6**) had no antifungal activity at concentrations lower than 100 μg/disc.

Emestrin (**3**) is a characteristic macrocyclic epidithiodioxopiperazine derivative and dethiosecoemestrin (**1**) is a trioxopiperazine derivative which has the same carbon skeleton as **3**. Thus, it seems likely that **1** was biosynthesized from **3** by desulfurization and oxidative degradation, involving the disconnection of the dioxopiperazine ring from the carbinol moiety. Compound **1** was easily hydrolyzed to give violaceic acid (**5**). The spectroscopic data of aurantioemestrin was closely similar to those of dethiosecoemestrin (**1**), so it is clear that

aurantioemestin is also related to **1** and **3**. The structure of aurantioemestrin is now under investigation.

Experimental

Melting points are uncorrected. IR and UV spectra were taken with a Hitachi 215 spectrophotometer and a Hitachi 124 spectrophotometer, respectively. MS were obtained on a JEOL JMS-D 300 spectrometer. ^{1}H-NMR and ^{13}C-NMR spectra were measured with a JEOL JNM-FX 100 spectrometer operating at 99.60 MHz (proton) and at 25.05 MHz (carbon-13), or with a JEOL JNM-GX 400 spectrometer at 399.78 MHz (proton) and at 100.43 MHz (carbon-13), using tetramethylsilane as an 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 ($^1J_{C,H}$). Optical rotations were measured with a JASCO DIP-181 spectrometer. CD curves were determined on a JASCO J-40 spectrophotometer. Medium-pressure liquid chromatography (MPLC) was performed with a Chemco Low-Prep 81-M-2 pump and glass column (i.d. 10 mm × 200 mm) of silica gel CQ-3 (30—50 μ; Wako).

Isolation of Dethiosecoemestrin (1) and Aurantioemestrin——The CH_2Cl_2 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) to give two fractions. The less polar fraction (160 mg) was rechromatographed on silica gel with $CHCl_3$–MeOH (200 : 1) followed by MPLC with the solvent system of benzene–acetone (20 : 1) to give aurantioemestrin (46 mg). The more polar fraction (500 mg) was purified by MPLC with the solvent system of $CHCl_3$ or $CHCl_3$–MeOH (200 : 1) to give dethiosecoemestrin (**1**) (214 mg).

Dethiosecoemestrin (**1**): Pale yellow crystalline powder with yellow fluorescence, mp 136 °C (dec.). IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 1715 (COO), 1680, 1670 (CON). UV λ_{max}^{MeOH} nm (log ε): 230 (4.26), 259 (4.17), 286 sh (4.00), 365 (3.83). EI-MS *m/z*: 486 (0.5%), 347 (1.8), 288 (100), 244 (18), 239 (59), 228 (18), 159 (29), 152 (32), 143 (27), 135 (54). FD-MS *m/z*: 556 (67%, M+NaH), 534 (100, M+2H), 533 (89, M+H), 532 (16, M^+), 288 (56). *Anal.* Calcd for $C_{27}H_{20}N_2O_{10}\cdot H_2O$: C, 58.91; H, 3.92; N, 5.05. Found: C, 58.99; H, 3.70; N, 4.77. ^{1}H-NMR ($CDCl_3$) δ: 3.292 (3H, s, NMe), 3.899 (3H, s, OMe), 4.968 (1H, dd, J=8.4, 1.5 Hz, 7-H), 5.386 (1H, ddd, J=8.2, 2.1, 1.5 Hz, 6-H), 5.530 (1H, dd, J=8.2, 2.4 Hz, 5a-H), 6.393 (1H, dd, J=8.4, 2.1 Hz, 8-H), 6.863 (1H, s, 11-H), 7.016 (1H, d, J=8.5 Hz), 7.019 (1H, d, J=2.4 Hz, 10-H), 7.135 (1H, d, J=8.3 Hz), 7.407 (1H, d, J=1.8 Hz), 7.571 (1H, dd, J=8.3, 1.8 Hz), 7.744 (1H, d, J=2.1 Hz), 7.942 (1H, dd, J=8.5, 2.1 Hz), 9.786 (1H, s, −CHO). ^{1}H-NMR (DMSO-d_6) δ: 3.10 (3H, s, NMe), 3.89 (3H, s, OMe), 4.97 (1H, dd, J=8.2, 1.5 Hz, 7-H), 5.22 (1H, ddd, J=8.4, 2.2, 1.5 Hz, 6-H), 5.45 (1H, dd, J=8.4, 2.3 Hz, 5a-H), 6.54 (1H, dd, J=8.2, 2.2 Hz, 8-H), 7.01 (1H, d, J=0.6 Hz, 11-H), 7.11 (1H, d, J=8.4 Hz), 7.18 (1H, d, J=2.3 Hz, 10-H), 7.27 (1H, d, J=2.1 Hz), 7.30 (1H, d, J=8.4 Hz), 7.46 (1H, d, J=2.1 Hz), 7.58 (1H, dd, J=8.4, 2.1 Hz), 7.95 (1H, dd, J=8.4, 2.1 Hz), 9.72 (1H, s, −CHO), 10.73 (1H, s, OH). $[\alpha]_D^{20}$ −213 ° (c=0.49, $CHCl_3$). CD (c=2.03 × 10^{-3}, MeOH) $[\theta]^{20}$ (nm): +6.0 × 10^4 (250) (positive maximum), +7.4 × 10^4 (263) (positive maximum), −1.3 × 10^4 (304) (negative maximum), −6.0 × 10^4 (362) (negative maximum). Compound **1** was slowly degradated to violaceic acid (**5**) in common organic solvents.

***O*-Acetyldethiosecoemestrin (2)**——Compound **1** (98 mg) was acetylated with acetic anhydride (0.7 ml) and pyridine (1.5 ml) at 5 °C overnight. The reaction mixture was poured into ice-water, and the resulting precipitate was collected and purified by chromatography with the solvent system of benzene–AcOEt (5 : 1) followed by MPLC with the solvent system of benzene–acetone (10 : 1) to give the monoacetate (**2**) (43 mg) as a pale yellow crystalline powder with yellow fluorescence, mp 140—142 °C. IR ν_{max}^{KBr} cm^{-1}: 1760, 1710 (COO), 1680 (CON), 1600. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1760, 1725 (COO), 1715, 1690, 1685 (CON), 1600. UV λ_{max}^{EtOH} nm (log ε): 222 sh (4.55), 254 (4.50), 287 sh (4.00), 367 (4.09). ^{1}H-NMR ($CDCl_3$) δ: 2.221 (3H, s, OAc), 3.314 (3H, s, NMe), 3.873 (3H, s, OMe), 5.005 (1H, dd, J=8.5, 1.8 Hz, 7-H), 5.483 (1H, ddd, J=8.1, 2.3, 1.8 Hz, 6-H), 5.563 (1H, dd, J=8.1, 2.4 Hz, 5a-H), 6.415 (1H, dd, J=8.5, 2.3 Hz, 8-H), 6.870 (1H, s, 11-H), 7.012 (1H, d, J=2.4 Hz, 10-H), 7.187 (2H, m), 7.622 (2H, m), 7.767 (1H, d, J=2.1 Hz), 8.057 (1H, dd, J=8.5, 2.1 Hz), 9.887 (1H, s, CHO). ^{1}H-NMR (benzene-d_6) δ: 1.821 (3H, s, OAc), 2.726 (3H, s, NMe), 3.121 (3H, s, OMe), 4.553 (1H, dd, J=8.3, 1.7 Hz, 7-H), 5.072 (1H, dd, J=8.2, 2.5 Hz, 5a-H), 5.363 (1H, ddd, J=8.2, 2.2, 1.7 Hz, 6-H), 5.790 (1H, dd, J=8.3, 2.2 Hz, 8-H), 5.984 (1H, s, 11-H), 6.028 (1H, d, J=2.5 Hz, 10-H), 6.470 (1H, d, J=8.5 Hz), 6.955 (1H, d, J=8.2 Hz), 7.327 (1H, dd, J=8.5, 1.7 Hz), 7.546 (1H, d, J=1.7 Hz), 8.284 (1H, dd, J=8.5 2.2 Hz), 8.312 (1H, d, J=2.2 Hz), 9.689 (1H, s, CHO). Compound **2** was easily degradated into **1** then **5** in common organic solvents.

Hydrogenation of Dethiosecoemestrin (1)——Compound **1** (130 mg) in MeOH (10 ml) was hydrogenated catalytically over Pt (50 mg) at room temperature in 4 h. After removal of the catalyst, the MeOH was evaporated off, and the reaction mixture was chromatographed on silica gel with $CHCl_3$–MeOH–AcOH (50 : 1 : 0.1) to afford violaceic acid (**5**) (25 mg). No other product was isolated.

Isolation of Paxilline (6)——The slightly less polar fraction than emestrin (**3**) obtained from the mycelial acetone extract of the above fungus was recrystallized from $CHCl_3$ to give paxilline (**6**) (395 mg) as colorless prisms, mp 253—255 °C (dec.). This compound was identified by comparison with a standard sample (IR, MS, and ^{1}H- and ^{13}C-NMR

spectra).

Antibacterial Activity Testing——The antibacterial activity was determined by the paper disc assay with *Escherichia coli* and *Bacillus subtilis* as test organisms. Bacteria were cultivated in Antibiotic Medium 3 (Difco). A two-day-old cultivated suspension (1 ml) and the above medium (19 ml) with 1.6% (w/v) agar (19 ml) were combined to prepare assay plates. The metabolites dissolved in acetone, MeOH, or tetrahydrofuran were charged onto paper discs (8 mm diameter) in the amounts mentioned in Table II, and placed on the assay plates. Zones of inhibition (mm in diameter) were recorded after a 24 h incubation at 37 °C.

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