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Isolation and Structures of Oxaphenalenone Dimers from *Talaromyces bacillosporus*

Mikio Yamazaki and Emi Okuyama[1)]

Research Institute for Chemobiodynamics, Chiba University[1)]

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Three new oxaphenalenone dimers, bacillosporins A—C, and one known xanthone carboxylic acid, pinselin, were isolated from a fungus, *Talaromyces bacillosporus*. Bacillosporin A (**1**) is an acetyl derivative of B (**2**). Bacillosporin A was transformed to xenoclauxin (**5**), a known oxaphenalenone dimer, by 2,3-dichloro-5,6-dicyanobenzoquinone (DDQ) oxidation. The hemiketal structure of bacillosporin C (**7**) was proposed on the basis of the spectral data for the compound itself and the hydrogenolysis product (**9**).

Keywords——bacillosporin; oxaphenalenone dimer; pinselin; *Talaromyces bacillosporus*; xanthone carboxylic acid; structure determination

Some novel oxaphenalenone dimers have already been isolated from the fungi *Penicillium duclauxi* (duclauxin, xenoclauxin, cryptoclauxin, *etc.*[2)]) and *Gilmaniella humicola* (gilmaniellin and dechlorogilmaniellin[3)]). We now report the isolation of three new yellow oxaphenalenone dimer pigments from *Talaromyces bacillosporus*. Pinselin, a hydroxy xanthone carboxylic acid, which had previously been isolated from the fungus *Penicillium amarum* and from the higher plant *Cassia occidentalis*, was also isolated together with the above three pigments.

Talaromyces bacillosporus (NHL 2660) was grown in a medium containing malt extract for 23 days at 25°. Mycelia were collected, dried and extracted with ether. The ether extract was defatted with *n*-hexane and fractionated on a silica gel column. Four pigments

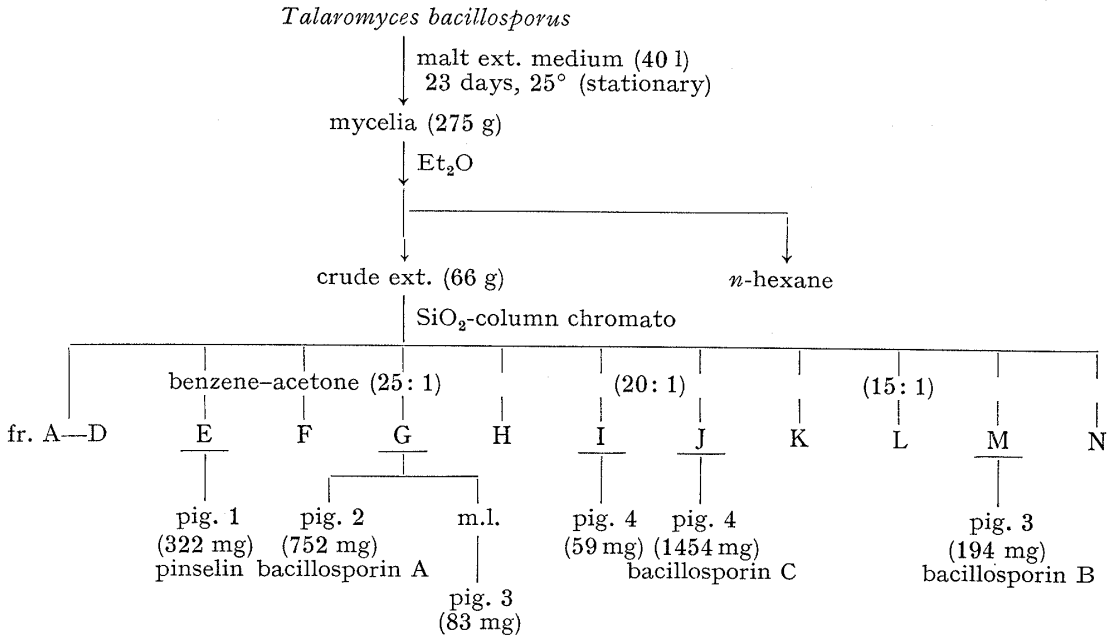


Chart 1

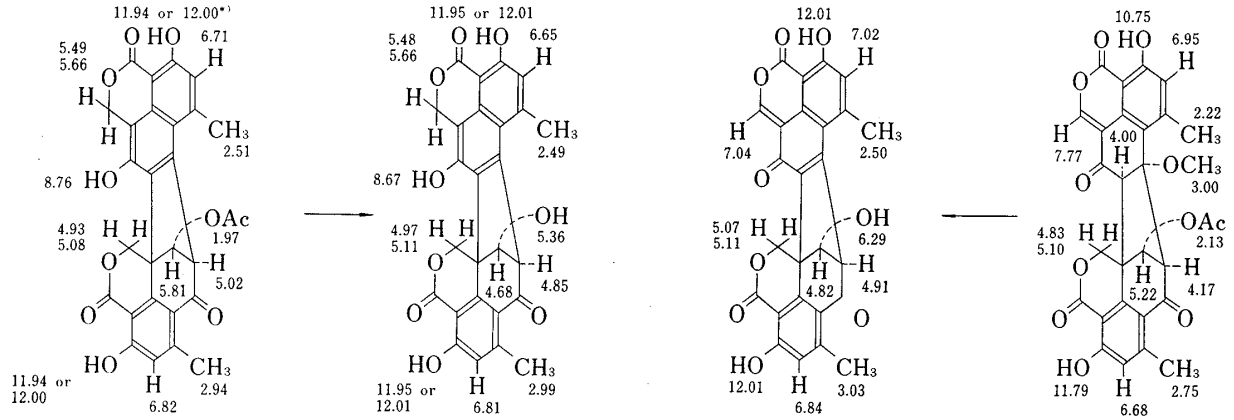
1) Location: *8-1, Inohana-1-chome, Chiba, 280, Japan.*
2) S. Shibata, Y. Ogihara, N. Tokutake, and O. Tanaka, *Tetrahedron Lett.*, **1965**, 1287; Y. Ogihara, O. Tanaka, and S. Shibata, *ibid.*, **1966**, 2867.
3) K.K. Chexal, C. Tamm, K. Hirotsu, and J. Clardy, *Helv. Chim. Acta*, **62**, 1785 (1972).

(pigs. 1—4) were isolated successively from fractions eluted with benzene and benzene–acetone as shown in Chart 1. Pigment 2—4 were found to be new metabolites and were designated as bacillosporins A—C (Bac-A—C).

Bacilloporin A(Bac-A), a pale yellow powder, mp 282—285° (decomp), $[\alpha]_D^{24}$ +484°, $C_{28}H_{20}O_{10}$ (M$^+$ *m/e* 516.1055, calcd 516.1055), was shown to be a phenolic compound by positive coloring in the $FeCl_3$ test. In the infrared (IR) spectrum, the presence of hydroxyl (3440 cm^{-1}) and carbonyl (1754 and 1672 cm^{-1}) groups was seen. In the proton nuclear magnetic resonance (PMR) spectrum, the presence of one CH_3CO- group was indicated by the appearance of a signal (3H, singlet) at 1.97 ppm. The signals at 11.94, 12.00 and 8.76 ppm in the PMR spectrum disappeared on treatment with D_2O, suggesting that two among the three hydroxyl groups formed hydrogen bondings. The hydroxyl group at 8.76 ppm was more easily methylated by diazomethane than the other two hydroxyl groups. The PMR spectrum of Bac-A further indicated that this compound contained two aromatic methyl groups (2.51 and 2.94 ppm) and two isolated aromatic protons (6.71 and 6.82 ppm). The signal of one aromatic proton (6.71 ppm) was sharpened by irradiation of one methyl signal (2.51 ppm) and that of the other proton (6.82 ppm) was sharpened by irradiation of the other methyl (2.94 ppm), indicating that a methyl group and an aromatic proton were possibly neighbors, in both cases. The appearance of four doublet protons as two AB quartets (at 4.93 and 5.08 ppm and at 5.49 and 5.66 ppm) suggested the presence of two -CH_2O- groups. A broad singlet (5.86 ppm in *d*-acetone) of a proton attached to a carbon bearing an acetoxyl group was shown to be sharpened by irradiation of the signal of a proton at 5.12 ppm (in *d*-acetone). This suggested that these two protons were also neighbors. The reason why the signals of these protons appeared as singlets may be that the angle between the two C-H bonds was nearly 90°. The carbon-13 nuclear magnetic resonance (CMR) data indicated the presence of three primary, two secondary, four tertiary and nineteen quaternary carbons. The signal of one quaternary carbon was observed at 49.5 ppm and the others appeared in the range below 90 ppm. The appearance of such a number of signals of quaternary (aromatic) carbons in the CMR spectrum suggested that Bac-A consisted of a condensed polyaromatic system.

Bac-A was deacetylated with 10% NaOH at room temperature to afford a a deacetyl derivative (2), yellow needles, mp 257—261°(decomp), $[\alpha]_D^{27}$ +512°, $C_{26}H_{18}O_9$ (M$^+$ *m/e* 474.0958, calcd 474.0951). The properties of the deacetyl derivative seemed to be identical to those of Bac-B, and these two compounds were shown to be identical by comparing the IR, PMR and mass spectra (MS). Bac-A was thus confirmed to be the acetyl derivative of Bac-B.

The resemblance of the PMR spectral data for Bec-B to those for neoclauxin (3), which

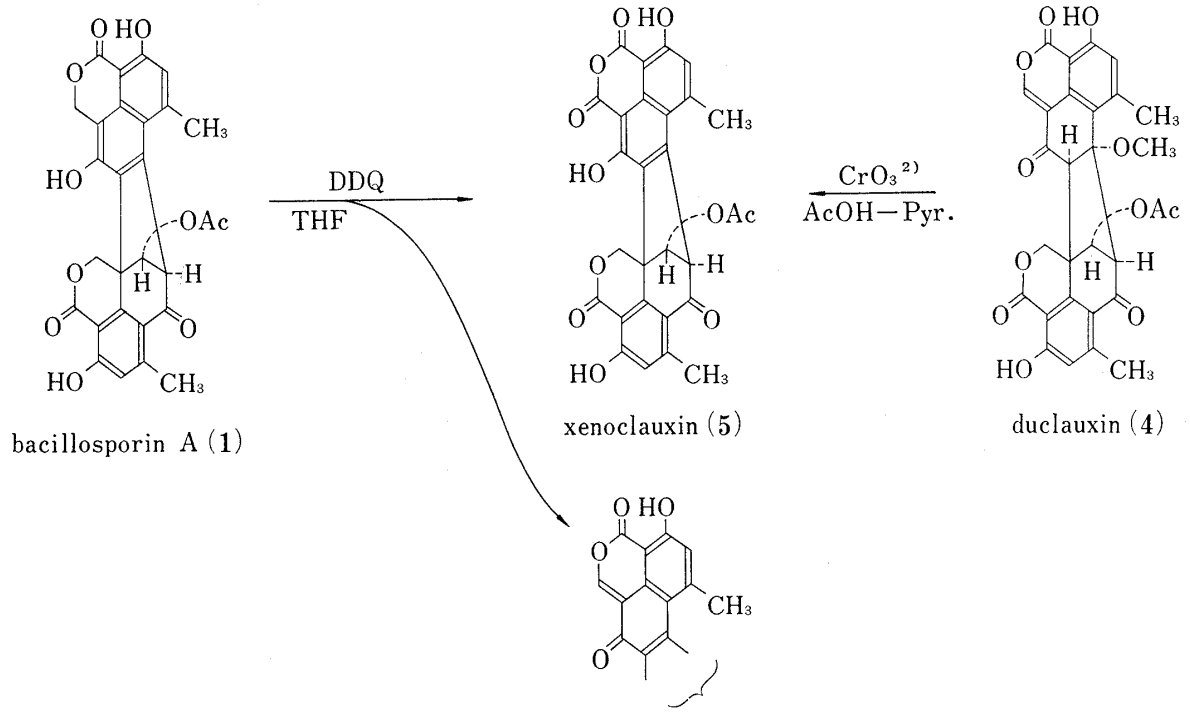


*) figures indicate the PMR data (δ value)

Chart 2

had previously been obtained from duclauxin (**4**) by alkali hydrolysis, was clear. The signal of the olefinic proton (7.04 ppm) which was observed in the PMR spectrum of neoclauxin disappeared in the spectrum of Bac-B, and instead, two doublets of AB quartet type (5.84 and 5.66 ppm) of $-CH_2O-$ and a singlet of phenolic OH (8.67 ppm) were observed. The optical rotatory dispersion (ORD) and circular dichroism (CD) spectra of these two compounds were also similar, suggesting that these two compounds retained similar stereostructures. The structure of Bac-B was thus proposed to be **2**.

On oxidation of Bac-A with DDQ in tetrahydrofuran, xenoclauxin (**5**) was obtained together with an unstable derivative (**6**). The structure of xenoclauxin has been established in relation to that of duclauxin (**4**), and the structure of duclauxin was determined by X-ray diffraction analysis.[4]



bacillosporin A (**1**) xenoclauxin (**5**) duclauxin (**4**)

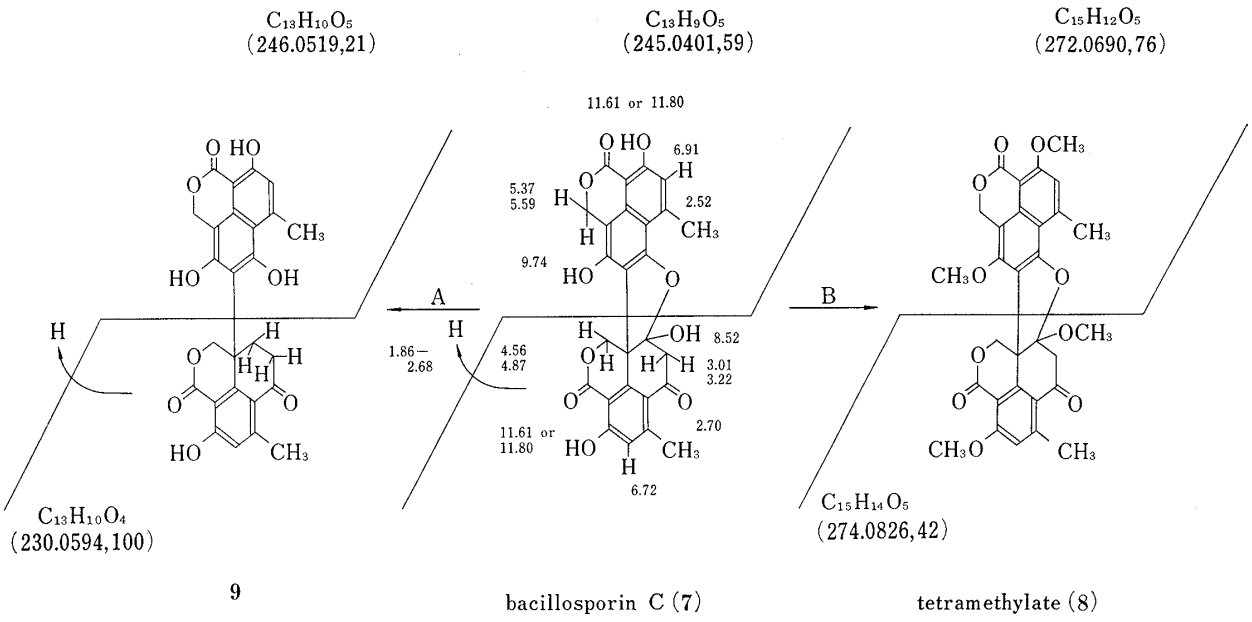
dehydro bacillosporin A (**6**)

Chart 3

Bacillosporin C(Bac-C), a pale yellow crystalline powder, mp>300°, $[\alpha]_D^{27.5}$ +451°, $C_{26}H_{18}O_{10}$ (M^+ *m/e* 490.0919, calcd 490.0899), exhibited an IR spectrum similar to that of Bac-B. On treatment with ammonia, Bac-C afforded a nitrogen-containing product, as in the case of duclauxin, indicating that Bac-C had a similar oxaphenalenone dimer structure. Bac-A also reacted with ammonia and afforded several reaction products on thin layer chromatography, (TLC) but these were not investigated further. An intense fragment peak of $C_{13}H_9O_5$ (*m/e* 245.0401, 59%) was observed in the mass spectrum of Bac-C. This showed that Bac-C easily gave the "monomer" fragments. The mass spectrum of the tetramethyl derivative of Bac-C (**8**), prepared by methylation of Bac-C with diazomethane, gave a fragment peak of $C_{15}H_{12}O_5$ (*m/e* 272.0690), supporting the above assumption. In the mass spectra of Bac-A and -B, no fragment peaks of such monomers were observed.

On catalytic hydrogenation over platinum, Bac-C afforded a hydrogenolysis product (**9**), pale yellow needles, mp 230—232°, $C_{26}H_{20}O_9$ (M^+ *m/e* 476.1102, calcd 476.1107). In the PMR spectrum of this product, multiplets of four protons appeared at 1.86—2.68 ppm, but

4) Y. Ogihara, Y. Iitaka, and S. Shibata, *Tetrahedron Lett.*, **1965**, 1289.



A; PtO_2/H_2 in 0.5% HCl–MeOH B; CH_2N_2 in Et_2O–THF
Chart 4

the two doublets of AB quartet type at 3.01 and 3.22 ppm which appeared in the spectrum of Bac-C were not observed. The fragment peaks of $C_{13}H_{10}O_4$ (*m/e* 230.0594, 100%) and $C_{13}H_{10}O_5$ (*m/e* 246.0519, 21%) were observed in the mass spectrum of this compound. It is known that the hydrogenolytic cleavage of carbon-hydroxyl bonds easily occurs in the hydrogenation of hemiacetals over platinum in an acidic alcoholic medium to afford ethers in high yield.[5] The formation of the hydrogenolysis product (**9**) during hydrogenation with a platinum catalyst was perhaps a result of the cleavage of C–O bonds. The structure of Bac-C was thus postulated to be **7**. Pigment 1, yellow prisms, mp 231—233°, $[\alpha]_D^{23}$ ±0, $C_{16}H_{12}O_6$ M^+ *m/e* 300), was found to be a phenolic compound (coloring with $FeCl_3$). In view of the spectral data, this pigment was judged to be a hydroxyxanthone, identical with pinselin. Pinselin is a xanthone carboxylic acid which has previously been isolated from the fungus *Penicillium amarum*[6] and the higher plant *Cassia occidentalis*,[7] as mentioned above. In the latter case, an incorrect structure was reported, and the pigment was designated as cassiolin, but the proposed structure was later found to be incorrect and was shown to be identical with that of pinselin.[8] Pigment 1 was confirmed to be identical to pinselin by direct comparison.

In connection with the biosynthesis of oxaphenalenones, several hypothetical pathways were considered.[9] Some results of tracer experiments with ^{14}C-labeled acetate and formate for the biosynthesis of duclauxin[10] and with ^{13}C-labeled acetate and malonate for deoxyherqueinone[11] have been reported.

The biological activity of the oxaphenalenones is not well understood, but we have found antibacterial activity of Bac-A against *Bacillus subtilis* and *Sarcina lutea*. No mutagenic

5) A.P.G. Kieboom and F. van Rantwijk, "Hydroengation and Hydrogenolysis," Delft Univ. Press 1977, p. 115.
6) H. Munakata, *Nogei Kagaku Kaishi*, **19**, 343 (1943); *idem*, *J. Biochem. Japan*, **40**, 451 (1953).
7) B.S. Ginde, B.D. Hosangadi, N.A. Kudav, K.V. Nayak, and A.B. Kulkarni, *J. Chem. Soc.* (*C*), **1970**, 1285.
8) C.E. Moppett, *J. Chem. Soc., Chem. Commun.*, **1971**, 423.
9) S. Shibata, *Chemistry in Britain*, **3**, 110 (1966); R. Thomas, *Pure and Applied Chemistry*, **34**, 515 (1973).
10) U. Sankawa, H. Taguchi, Y. Ogihara, and S. Shibata, *Tetrahedron Lett.*, **1966**, 2883.
11) T.J. Simpson, *J. Chem. Soc.* (*C*), **1976**, 258; **1979**, 1233.

activity was observed for these metabolites of *Talaromyces bacillosporus* in mutation tests with *Salmonella typhimurium*.

Experimental

All melting points are uncorrected. Optical rotation was measured on a Yanagimoto Or-50 automatic polarimeter. Ultraviolet (UV), IR, PMR and CMR spectra were measured with Hitachi 323, Hitachi EPI-G3, JEOL JNM PS-100 and JEOL JNM PFT-100 machines, respectively (chemical shifts are shown in ppm from Me_4Si added as an internal standard). MS spectra were measured on a JEOL JMS 01SG-2 machine equipped with a JMA 2000 mass data analysis system. CD spectra were recorded on a JASCO ORD/CD J-20 unit. TLC was carried out on 0.25 mm precoated silica gel 60 F_{254} plates (Merck) and the plates used for prep. TLC were coated with Merck Silica gel GF_{254} or HF_{254} (0.5 mm thickness).

Isolation of Pigments——*Talaromyces bacillosporus* (NHL 2660) was grown in stationary culture in 800 ml Roux bottles, each containing 200 ml of culture medium composed of 2% malt extract, 2% dextrose and 0.1% peptone at 25° for 23 days. The mycelia (275 g, dry) were extracted 4 times with ether (2 l each) by refluxing for 2—3 hr. The hexane-insoluble part (66 g) of the extract was subjected to silica gel column chromatography (Wakogel C-200, 1 kg). Pigment 1 (pinselin) (322 mg, 0.49%) and pigment 2 (bacillosporin A) (752 mg, 1.1%) were eluted with a mixture of acetone–benzene (1: 25). Pigment 4 (bacillosporin C) (1513 mg, 2.3%) was eluted with acetone–benzene (1: 25—1: 20). Pigment 3 (bacillosporin B) (277 mg, 0.42%) was eluted with acetone–benzene (1: 15) Pigment 3 was further obtained from the mother liquor after the isolation of pigment 2

Pigment 1 (Pinselin): Yellow prisms from CH_2Cl_2–MeOH, mp 231—233°, $[\alpha]_D^{23}$ 0° (0.101, acetone). *Anal.* Calcd for $C_{16}H_{12}O_6$: C, 64.00; H, 4.03; N, 0.00. Found: C, 63.98; H, 3.96; N, 0.08. IR ν_{max}^{KBr} cm^{-1}: 3330, 3260, 3035, 2985, 2935, 1741, 1648, 1608, 1589, 1500, 1282, 1226, 1204, 1018. UV λ_{max}^{MeOH} nm (ε): 238 (26900), 257 (sh, 28200), 265 (35200), 295 (9500), 387 (6300). MS *m/e* (%): 300 (M^+, 31), 268 (100), 240 (12). MS *m/e*: 300.0716 (Calcd for $C_{16}H_{15}O_6$: 300.0634). PMR (in acetone-d_6) δ: 2.38 (3H, s), 3.90 (3H, s), 6.52 (1H, s), 6.71 (1H, s), 7.45 (2H, s), 9.07 (1H, br.s, disappeared with D_2O), 12.12 (1H, s, disappeared with D_2O). PMR (in THF-d_8) δ: 2.35 (3H, s), 3.89 (3H, s), 6.49 (1H, s), 6.62 (1H, s), 7.23 (1H, d, J=9), 7.35 (1H, d, J=9), 8.90 (1H, br.s, disappeared with D_2O), 12.26 (1H, s, disappeared with D_2O). CMR (in pyr.-d_5) δ: 22.2 (q), 52.5 (q), 107.0 (s), 107.5 (d), 111.2 (d), 118.6 (s), 119.1 (s), 120.1 (d), 125.5 (d), 149.1 (s), 152.2 (s), 156.1 (s), 161.7 (s), 167.3 (s), 168.1 (s), 181.2 (s).

Pigment 2 (Bacillosporin A): Pale yellow powder from acetone–$CHCl_3$, mp 282—285° (dec.), $[\alpha]_D^{24}$ +484° (0.583, acetone). IR ν_{max}^{KBr} cm^{-1}: 3440, 1754, 1672, 1604, 1586, 1567, 1218, 1048. UV λ_{max}^{MeOH} nm (ε): 233 (40400), 242 (sh, 38700), 259 (sh, 30300), 278 (sh, 17700), 322 (8900), 354 (sh, 6500), 376 (sh, 3500), 415 (1400). MS *m/e* (%): 516 (M^+, 100), 474 (72), 456 (10), 426 (10), 416 (10), 399 (9). PMR (in acetone-d_6) δ: 2.04 (3H, s), 2.52 (3H, s), 2.98 (3H, s), 5.11 (1H, d, J=12), 5.23 (1H, d, J=12), 5.12 (1H, s), 5.63 (1H, d, J=16), 5.82 (1H, d, J=16), 5.86 (1H, s), 6.73 (1H, s), 6.81 (1H, s), 8.58 (1H, br.s, disappeared with D_2O), 11.81 (1H, s, disappeared with D_2O), 11.87 (1H, s, disappeared with D_2O). PMR (in THF-d_8) δ: 1.97 (3H, s), 2.51 (3H, s), 2.94 (3H, s), 4.93 (1H, d, J=13), 5.02 (1H, s), 5.08 (1H, d, J=13), 5.49 (1H, d, J=16), 5.66 (1H, d, J=16), 5.81 (1H, s), 6.71 (1H, s), 6.82 (1H, s), 8.76 (1H, disappeared with D_2O), 11.94 (1H, disappeared with D_2O), 12.00 (1H, disappeared with D_2O). CMR (in acetone-d_6) δ: 20.7 (q), 23.7 (q), 25.1 (q), 49.5 (s), 62.7 (d), 67.5 (t), 70.1 (t), 86.8 (d), 98.2 (s), 104.5 (s), 111.2 (s), 117.8 (s), 120.3 (s),, 120.7 (d), 121.1 (d), 132.6 (s), 134.5 (s), 137.7 (s), 146.8 (s), 147.5 (s), 148.6 (s), 154.1 (s), 163.3 (s), 165.1 (s), 168.7 (s), 170.0 (s), 170.7 (s), 191.7 (s). CD (c=0.000368 mol/l, THF) $[\theta]^{22}$ (nm): +2700 (sh, 385), +7500 (max, 368), +6800 (min, 362), +14600 (sh, 345), +22100 (max, 331), 0 (317), −51300 (min, 298), 0 (282), +14300 (sh, 275), +109000 (max, 259), +97800, (min, 255), +182000 (max, 239), 0 (230), −86900 (min, 222).

Pigment 3 (Bacillosporin B): Yellow crystalline powder from acetone–MeOH, mp 257—261° (dec.), $[\alpha]_D^{27}$ +512° (0.144, THF). *Anal.* Calcd for $C_{26}H_{18}O_9 \cdot 1/4H_2O$: C, 65.21; H, 3.89; N, 0.00. Found: C, 65.17; H, 4.11; N, 0.14. IR ν_{max}^{KBr} cm^{-1}; 3435, 1667, 1600, 1566, 1266, 1215. UV λ_{max}^{MeOH} nm (ε); 233 (43900), 242 (sh, 41100), 258 (sh, 33400), 275 (sh, 18300), 324 (9200), 353 (sh, 7200), 373 (sh, 4200), 410 (sh, 1200). MS *m/e* (%): 474 (M^+, 100), 456 (8), 444 (11), 426 (10), 416 (17), 400 (7). MS *m/e*: 474.0958 (Calcd for $C_{26}H_{18}O_9$: 474.0951). PMR (in THF-d_8) δ: 2.49 (3H, s), 2.99 (3H, s), 4.68 (1H, br.s), 4.85 (1H, s), 4.97 (1H, d, J=12), 5.11 (1H, d, J=12), 5.36 (1H, br.s, disappeared with D_2O), 5.48 (1H, d, J=15), 5.66 (1H, d, J=15), 6.65 (1H, s), 6.81 (1H, s), 8.67 (1H, br.s, disappeared with D_2O), 11.95 (1H, s, disappeared with D_2O), 12.01 (1H, br.s, disappeared with D_2O), CD (c=0.000266 mol/l, THF) $[\theta]^{22}$ (nm): +3800 (sh, 385), +13200 (max, 367), +13200 (min, 363), +31000 (sh, 346), +48900 (max, 328), 0 (315), −111000 (min, 296), 0 (280), +31000 (sh, 274), +222000 (sh, 257), +421000 (max, 240), 0 (229), −192000 (min, 222) Neoclauxin CD (c=0.000265 mol/l, THF) $[\theta]^{29}$ (nm): 0 (390), +600 (max, 385), 0 (382), −800 (min, 378), 0 (376), +7600 (max, 368), +5100 (min, 362), +24700 (sh, 347), +50600 (max, 326), 0 (314), −123000 (min, 297), 0 (280), +32100 (sh, 275), +202000 (sh, 255), +366000 (max, 241), 0 (230), −172000 (min, 220).

Pigment 4 (Bacillosporin C): Pale yellow crystalline powder from THF–MeOH, mp>300°, $[\alpha]_D^{28}$ +451° (0.200, acetone). *Anal.* Calcd for $C_{26}H_{18}O_{10}$: C, 63.67; H, 3.70; N, 0.00. Found: C, 63.68; H, 3.64; N, 0.04 IR ν_{max}^{KBr} cm^{-1}: 3450, 1667, 1619, 1572, 1457, 1361, 1304, 1170, 1002. UV λ_{max}^{MeOH} nm (ε): 221 (sh, 42600),

233 (sh, 48600), 241 (50100), 270 (sh, 30300), 275 (30800), 317 (sh, 8400), 345 (6400), 361 (sh, 5700), 422 (sh, 900). PMR (in THF-d_8) δ: 2.62 (3H, s), 2.80 (3H, s), 2.97 (1H, d, J=16), 3.32 (1H, d, J=16), 4.69 (1H, d, J=11), 4.91 (1H, d, J=11), 5.40 (1H, d, J=14), 5.63 (1H, d, J=14), 6.70 (1H, s), 6.88 (1H, s), 7.71 (1H, br.s, disappeared with D_2O), 8.70 (1H, br.s, disappeared with D_2O), 11.76 (1H, s, disappeared with D_2O), 11.89 (1H, s, disappeared with D_2O). PMR (in DMSO-d_6) δ: 2.52 (3H, s), 2.70 (3H, s), 3.01 (1H, d, J=15), 3.22 (1H, d, J=15), 4.56 (1H, d, J=11), 4.87 (1H, d, J=11), 5.37 (1H, d, J=14), 5.59 (1H, d, J=14), 6.72 (1H, s), 6.91 (1H, s), 8.52 (1H, s, disappeared with D_2O), 9.74 (1H, br.s, disappeared with D_2O), 11.61 (1H, s, disappeared with D_2O), 11.80 (1H, s, disappeared with D_2O). CMR (in THF-d_8) δ: 23.5 (q, 2), 49.6 (t), 49.9 (s), 67.3 (t), 74.5 (t), 97.5 (s), 102.9 (s), 109.9 (s), 110.3 (s), 112.4 (s), 114.5 (s), 118.2 (d), 120.7 (d), 122.0 (s), 133.2 (s), 145.2 (s), 146.5 (s), 150.0 (s), 150.8 (s), 156.3 (s), 164.4 (s), 164.8 (s), 170.2 (s), 170.4 (s), 193.4 (s). CD (c=0.000506 mol/l, THF) $[\theta]^{22}$ (nm): +1500 (sh, 381), +8200 (max, 347), +7900 (min, 344), +13300 (max, 321), 0 (307), −9400 (min, 289), 0 (282), +33600 (sh, 275), +405000 (max, 239), 0 (226), −263000 (min, 219).

Methylation of Bacillosporin A——A mixture of bacillosporin A (53 mg) and CH_2N_2-containing ether was stirred for 40 min at room temperature. The reaction solvent was evaporated off, and the residue was subjected to prep. TLC (benzene: acetone=6: 1) to give the monomethylated compound (41 mg) and the dimethylated compound (6 mg), which were further purified by recrystallization from CH_2Cl_2–MeOH.

Monomethylbacillosporin A: Colorless needles, mp 198—201°, $[\alpha]_D^{23}$ +620° (0.115, $CHCl_3$). *Anal.* Calcd for $C_{29}H_{22}O_{10}\cdot 1/2H_2O$: C, 64.56; H, 4.30; N, 0.00. Found: C, 64.62; H, 4.04; N, 0.05. IR ν_{max}^{KBr} cm^{-1}: 3450, 1751, 1676, 1602, 1570, 1219, 1047. UV λ_{max}^{MeOH} nm (ε): 229 (35900), 247 (41600), 255 (sh, 39400), 280 (sh, 19600), 308 (11300), 317 (11300), 350 (sh, 5500), 371 (sh, 3900). MS *m/e* (%): 530 (M^+, 100), 488 (100), 460 (10), 427 (11), 399 (12), 256 (11), 239 (11), 226 (12), 215 (10). PMR (in $CDCl_3$) δ: 2.06 (3H, s), 2.59 (3H, s), 3.02 (3H, s), 3.81 (3H, s), 4.89 (1H, d, J=12), 5.05 (1H, s), 5.07 (1H, d, J=12), 5.51 (1H, d, J=16), 5.79 (1H, d, J=15), 5.79 (1H, s), 6.79 (1H, s), 7.00 (1H, s), 11.76 (2H, s, disappeared with D_2O).

Dimethylbacillosporin A: Colorless needles, mp 202—208°. IR ν_{max}^{KBr} cm^{-1}: 3425, 1730, 1676, 1589, 1550, 1220, 1026. UV λ_{max}^{MeOH} nm (ε): 231 (sh, 37300), 246 (41900), 253 (sh, 38500), 278 (24400), 305 (sh, 11500), 316 (sh, 10500), 360 (sh, 4600), 373 (sh, 4100). MS *m/e* (%): 544 (M^+, 100), 502 (80), 484 (58), 472 (18), 453 (11), 441 (11). MS *m/e* (%): 544.1352 (Calcd for $C_{30}H_{24}O_{10}$: 544.1368). PMR (in $CDCl_3$) δ: 2.05 (3H, s), 2.65 (3H, s), 3.04 (3H, s), 3.82 (3H, s), 4.01 (3H, s), 4.78 (1H, d, J=12), 4.94 (1H, d, J=12), 5.04 (1H, s), 5.61 (1H, d, J=16), 5.70 (1H, s), 5.79 (1H, d, J=16), 6.79 (1H, s), 7.02 (1H, s), 11.80 (1H, s, disappeared with D_2O).

Deacetylation of Bacillosporin A——A mixture of bacillosporin A (32 mg) and 10% NaOH solution (0.5 ml) was stirred for 1 hr at room temperature. The resulting orange solution was acidified under ice-cooling and extracted with ether. Deacetylation of bacillosporin A proceeded smoothly. The deacetyl product was purified by prep. TLC ($CHCl_3$: MeOH=10: 1) and recrystallized from CH_2Cl_2–MeOH to give a faintly yellow crystalline powder, mp 257—261° (dec.), which was identical with bacillosporin B as judged by TLC behavior ($CHCl_3$: MeOH=10: 1), and IR, PMR and MS spectra.

Conversion of Bacillosporin A into Xenoclauxin——DDQ (40 ml) was added to a solution of bacillosporin A (40 mg) in THF (1 ml) and stirred for 15 min at room temperature. The reaction solution was subjected to prep. TLC ($CHCl_3$: MeOH=15: 1). One of the oxidized compounds (15 mg) crystallized from CH_2Cl_2–MeOH was identical with xenoclauxin as judged by TLC behavior (oxalic acid-impregnated plates, benzene: acetone=10: 1, CH_2Cl_2: AcOEt=20: 1, benzene: AcOEt=30: 1), mixed fusion, and IR, PMR and MS spectra. Another compound (19 mg) obtained was very unstable and could not be purified completely. The properties of the latter compound were as follows; pale yellow amorphous. IR ν_{max}^{KBr} cm^{-1}: 3440, 1750, 1667, 1603, 1383, 1222, 1050. UV λ_{max}^{EtOH} nm (ε): 242, 280 sh, 320, 413. PMR (in DMSO-d_6) δ: 2.00 (3H, s), 2.44 (3H, s), 2.92 (3H, s), 4.66—5.20 (3H, br), 5.72 (1H, s), 6.70 (1H, s), 6.88 (1H, s), 7.16 (1H, s).

Methylation of Bacillosporin C——An excess of ethereal CH_2N_2 (1.5 ml) was added to bacillosporin C (64 mg) dissolved in THF (3 ml) and the solution was allowed to stand in a refrigerator for 16 hr. The solvent was evaporated off and the products, tri- and tetramethyl ether, were isolated by prep. TLC (benzene: acetone =6: 1) and purified by recrystallization from MeOH.

Trimethylbacillosporin C: Pale yellow granules, mp 293—295° (dec.). IR ν_{max}^{KBr} cm^{-1}: 3500, 1743, 1668, 1588, 1292, 1031. UV λ_{max}^{MeOH} nm (ε): 218 (sh, 33900), 238 (43900), 267 (sh, 29300), 275 (33700), 312 (7300), 360 (4900). MS *m/e* (%): 532 (M^+, 100). MS *m/e*: 532.1369 (Calcd for $C_{29}H_{24}O_{10}$: 532.1369). PMR (in pyr.-d_5) δ: 2.76 (6H, s), 3.06 (3H, s), 3.54 (2H, s), 3.61 (3H, s), 3.95 (3H, s), 4.76 (1H, d, J=11), 4.89 (1H, d, J=11), 5.43 (1H, d, J=15), 5.63 (1H, d, J=15), 6.92 (1H, s), 7.08 (1H, s).

Tetramethylbacillosporin C: Pale yellow granules, mp 185—187°. IR ν_{max}^{KBr} cm^{-1}: 3500, 1730, 1673, 1613, 1587, 1295, 1238, 1097, 1028. UV λ_{max}^{MeOH} nm (ε): 227 (41800), 240 (45500), 270 (sh, 40400), 275 (42100), 312 (7600), 340 (5000), 366 (5600). MS *m/e* (%): 546 (M^+, 100), 532 (30), 515 (10), 501 (14), 485 (15), 469 (12), 453 (13), 274 (42), 273 (42), 272 (76), 258 (18), 243 (28), 229 (21), 215 (12), 201 (13). MS *m/e*: 546.1518 (Calcd for $C_{30}H_{26}O_{10}$: 546.1524), 274.0826 (Calcd for $C_{15}H_{14}O_5$: 274.0841), 273.0767 (Calcd for $C_{15}H_{13}O_5$: 273.0762), 272.0690 (Calcd for $C_{15}H_{12}O_5$: 272.0685). PMR (in $CDCl_3$) δ: 2.70 (3H, s), 2.88 (3H, s), 3.13 (3H, s), 3.30 (2H, s), 3.65 (3H, s), 4.06 (3H, s), 4.09 (3H, s), 4.45 (1H, d, J=11), 4.71 (1H, d, J=11), 5.18 (1H, d, J=15), 5.51 (1H, d, J=15), 6.94 (1H, s), 7.02 (1H, s).

Catalytic Hydrogenation of Bacillosporin C——Bacillosporin C (50 mg) was stirred in a 0.5% HCl–MeOH solution (4.5 ml) under H_2 gas over PtO_2 for 80 min at room temperature. The product (33 mg) was purified by prep. TLC ($CHCl_3$: MeOH=10:1) and recrystallized from EtOH to give pale yellow needles, mp 230—232°. IR ν_{max}^{KBr} cm^{-1}: 3410, 1660, 1618, 1317, 1217. UV λ_{max}^{MeOH} nm (ε): 219 (46600), 254 (sh, 20300), 276 (17600), 327 (9200), 364 (sh, 5400). MS *m/e* (%): 476 (M^+, 15), 445 (11), 246 (21), 245 (25), 244 (24), 231 (16), 230 (100), 229 (51), 218 (10), 216 (11), 201 (76). FD-MS *m/e* (%): 476 (M^+, 100). MS *m/e*: 476.1102 (Calcd for $C_{26}H_{20}O_9$: 476.1107), 246.0519 (Calcd for $C_{13}H_{10}O_5$: 246.0526), 245.0458 (Calcd for $C_{13}H_9O_5$: 245.0451), 244.0340 (Calcd for $C_{13}H_8O_5$: 244.0370), 230.0594 (Calcd for $C_{13}H_{10}O_4$: 230.0579). PMR (in DMSO-d_6) δ: 1.86 (1H, m), 2.32 (2H, m), 2.68 (1H, m), 2.20 (3H, s), 2.77 (3H, s), 4.64 (1H, d, J=12), 4.87 (1H, d, J=12), 5.41 (1H, d, J=15), 5.62 (1H, d, J=15), 6.75 (2H, s), 7.83 (1H, s), 9.69 (1H, s, disappeared with D_2O), 11.21 (1H, s, disappeared with D_2O), 11.84 (1H, s, disappeared with D_2O).

NH_3 Treatment of Bacillosporin C——A mixture of bacillosporin C (37 mg) and 28% NH_3 solution (3 ml) was stirred at room temperature for 5 min and extracted with AcOEt after acidification. The extract was purified by prep. TLC ($CHCl_3$: MeOH=10:1) and recrystallized from acetone–H_2O to obtain yellow needles (24 mg), mp 272° (dec.). IR ν_{max}^{KBr} cm^{-1}: 3425, 1667, 1637, 1561, 1449, 1253. UV λ_{max}^{MeOH} nm (ε): 214 (34700), 225 (33600), 235 (34200), 270 (sh, 18200), 314 (sh, 10600), 324 (13000), 347 (7200), 362 (10300), 400 (sh, 5800), 423 (7200), 442 (sh, 5900). MS *m/e* (%): 487 (M^+, 5), 255 (16), 243 (100), 232 (53), 216 (17), 214 (15). FD-MS *m/e* (%): 487 (M^+, 100). PMR (in DMSO-d_6) δ: 2.50 (3H, s), 2.72 (3H, s), ~3.2, 4.67 (1H, d, J=11), 4.89 (1H, d, J=11), 6.84 (1H, s), 6.86 (1H, s), 8.13 (1H, s), 11.80 (1H, s, disappeared with D_2O), 13.10 (1H, s, disappeared with D_2O).

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