Stereochemistry of Cervicarcin

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The relative stereochemistry of cervicarcin, an antitumor antibiotic, was determined as shown in 1, which represents the absolute stereochemistry also.

Cervicarcin is an antitumor antibiotic produced by Streptomyces ogaensis. We have shown the plane structure. We wish now to present evidence which deduced the relative stereochemistry of cervicarcin as 1. That 1 represents the absolute stereochemistry also has been communicated already.

Cervilactone (4), a degradation product of methylcervicarcin (2), gave useful informations on the stereochemistry of 1, being prepared from 2 by two steps. Namely, 2 was oxidized with sodium metaperiodate to cervic acid (3). 3 was converted into 4 upon heating in a boiling pH 3 aqueous solution. The latter conversion occurred in high yield when pH of the solution was adjusted to 3, whereas a pH 1 aqueous solution or 1N HCl did not cause it but 3 was recovered. This indicates that the oxirane ring on the C-4a, -9a juncture was opened to a hydroxyl group by attack with a partially ionized carboxylate anion from back-side, and not such the case that the oxirane ring was first hydrolyzed to 1,2-glycol by H+ catalysis, followed by subsequent lactonization. Therefore, the 4a-OH configuration of 4 should be the same as that of the oxirane ring of 3. The NMR spectrum (CDCl3) of 4 is that δ 5.50 (s, 1-H), 3.33 (q, J=8.0 Hz, 2-H), 1.48 (d, J=8.0 Hz, 11-CH3), 6.27 and 6.46 (each s, 4-H and 10-H, or vice versa). In order to construct a γ-lactone ring with simultaneous retention of a five-membered hemiacetal ring, 1-H must be situated on the same side as 4a-OH, hence, it in 3 on the same side as the oxirane ring. Now, we postulate the C-4a, -9a oxirane ring on β-side tentatively, and try to determine other asymmetric centers in relation to this β-oxirane ring. In this way, 1-H of 3 was on β-side, hence, it must be α in 1. 1-β-H of 4 appeared as a singlet in the NMR spectrum, showing that its dihedral angle with vicinal 2-H should be ca. 90°. Only α-H on C-2 satisfied the required angle from a dreiding model consideration, therefore, 11-CH3 must be on β-side in 4 and α in 1.

4 was methylated with MeOH-HCl to methyl-acetal (5), mp 187°C, which was treated with acetone-HCl or paraaldehyde-H2SO4 to afford respective products of an acetonide (6), mp 161°C, and an ethylidene

No OH absorption in their infrared spectra indicated that the ketal and the acetal formed with the vicinal glycol on C-4a and -10. As 4a-β-OH has to take an axial conformation in these rigid systems, 10-OH must be on the same side as 4a-β-OH to form the ketal derivatives, being concluded to be on β-side. The relative configuration of 4 was thus established, which deduced the relative stereochemistry of C-1, C-2 and C-10 of 1 related to the C-4a, -9a oxirane ring.

4-β-OH was shown as follows. Ketalization of 2 with cyclohexanone in refluxing benzene with p-toluenesulfuric acid for 4 hr afforded 8, mp 179°C. On further refluxing, 8 rearranged to 10, mp 137°C. Upon acetylation, 8 and 10 gave monoacetate (9), mp 129°C and diacetate (11), mp 231°C, respectively. The rearranged product (10) was determined by spectroscopic analyses. Namely, 11 showed characteristic carbonyl bands at ν\text{KBr} 1778 cm\(^{-1}\) (C=O of tetrahydrofuranone), 1750 (acetate), 1698 cm\(^{-1}\) (9-aryl ketone) and no OH; NMR (CDCl\(_3\)) \(\delta\) 2.06 and 2.12 (two CH\(_3\)COO), 1.48 (d, \(J=6.0\ Hz\), 15-CH\(_3\)), and 5.21 (d, \(J=9.3\ Hz\), 13-H). 14-H was recognized as a multiplet at \(\delta\ ca.\ 4.0\) being overlapped with the signals of 4-H (\(\delta\ 4.03\)) and 5-OCH\(_3\) (\(\delta\ 3.83\)). 10 exhibited 1-H at \(\delta\ 4.52\) (d, \(J=3.6\ Hz\)) which shifted in 11 to \(\delta\ 5.95\), whereas two singlet protons on both C-4 (\(\delta\ 4.03\)) and C-10 (\(\delta\ 5.51\)) in the former did not shift significantly even after acetylation. From these results, ketalization was shown to occur at 4- and 10-diols, and for making it possible 4-OH should be in cis relation to 10-β-OH. Similar rearrangement catalyzed by acid was conducted on a naphthalenic compound (12), a hydrogenolyzed product of 2 with palladium on carbon, affording 13, mp 227°C, which exhibited a carbonyl band of tetrahydrofuranone at ν\text{KBr} 1770 cm\(^{-1}\) and the signals of 11-, 15-CH\(_3\) and two OCH\(_3\) at \(\delta\) (DMSO-D\(_6\)) 0.97 (d, \(J=6.8\ Hz\)), 1.36 (d, \(J=5.0\ Hz\)), 3.84 (s) and 3.98 (s), respectively. Two sec. hydroxyl protons at C-4 and -13 appeared as a doublet at \(\delta\ 5.73\) (\(J=10\ Hz\)) and 5.97 (\(J=6.3\ Hz\)). Both signals disappeared by adding D\(_2\)O. A doublet of 4-H was at \(\delta\ 4.62\) (\(J=10\ Hz\)) being changed into a singlet with D\(_2\)O addition. 13-H which hidden in OCH\(_3\) (\(\delta\ 3.98\)) could be observed after addition of D\(_2\)O.

Further evidence on 4-β-OH as well as clarification of the 3-OH configuration were
Stereochemistry of Cervicarcin was carried out as follows. 2 was treated with paraaldehyde-H$_2$SO$_4$ to produce a diethylidene derivative (14), mp 265°C, $\nu$KBr 1736 (12-ketone), 1695 (9-aryl ketone) and no OH; NMR (CDCl$_3$) $\delta$ 1.42 (d, $J$=5.4 Hz, two CH$_3$ of diethylidene), 5.21 and 5.28 (two q. partially overlapped with each other, $J$=5.4 Hz, two methines of diethylidene). Simultaneous formation of the ethylidene bridges on the two diol pairs at both C-1, -3 and C-4, -10 is only possible as such the conformation of 1 that the former diol are diaxial and the latter are diequatorial as depicted in 14. Thus, conclusive evidence on 3-$\beta$ and 4-$\beta$-OH was obtained.

The side chain trans-epoxide was shown from the coupling constant of 13-H with 14-H to be 2.0 Hz,$^6$ although its absolute configuration is not clear.

The relative stereochemistry of cervicarcin has been shown as 1, except the side chain epoxide. That 1 also indicates the absolute stereochemistry has been shown$^{11}$ on the basis of the absolute configuration of 4-sec. hydroxyl of a naphthalenic compound (12) which had been determined by application of the aromatic chirality method.

**EXPERIMENTAL**

Melting points were uncorrected. Infrared spectra were measured with Shimazu IR-27 G, and NMR spectra with Varian A-60 and JNM-C-60, $-4 H-100$ spectrometers with tetramethylsilane as an internal standard.

*Isopropylidene cervilactone methyl acetal (6).* Cervilactone methyl acetal (5) (132 mg) dissolved into dry acetone (30 ml) containing 3% HCl was kept standing at 0°C overnight. The anhydrous potassium carbonate was added and stirred for neutralization of the acid. The precipitates were filtered off, and the filtrate was

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evaporated in vacuo to dryness. The oily residue was purified through a column chromatography on silicic acid. Isopropylidene compound (6) was eluted out with 10% ethyl acetate in benzene. The eluate was after evaporating the solvent dissolved into hot petroleum ether, and then precipitated by cooling. Methyl-acetal (5) was recovered from the column by eluting with 20% ethyl acetate in benzene. 6 thus obtained was a colourless viscous oil, which solidified upon drying at 80°C in vacuo for 2 hr.

6, mp 150–161°C; \( \nu_{KBr} \) no OH, 2940 (split), 1795, 1720, 1580, 1475, 1383, 1295, 1272, 1215, 1175, 1150, 1125, 1095, 1060, 970, 835 and 815 cm\(^{-1}\). Anal. Found: C, 61.49; H, 5.67. Calcd. for C\(_{20}\)H\(_{22}\)O\(_8\): C, 61.53; H, 5.68%.

Ethylidene cervilactone methyl acetal (7). The mixture of cervilactone methyl acetal (5) (100 mg), paraldehyde (4 ml) and cone. sulfuric acid (1 drop) was kept standing at room temperature overnight. Ethyl acetate (50 ml) was added to the reaction mixture, which was after washing with saturated aqueous sodium bicarbonate and water evaporated in vacuo to dryness. The oily residue was chromatographed on silicic acid. 7 was eluted from the column with 10% ethyl acetate in benzene, and solidified upon drying at 80°C for 4 hr.

7, mp 89–94°C; \( \nu_{KBr} \) no OH, 2930, 1795, 1715, 1580, 1475, 1440, 1328, 1295, 1270, 1215, 1142, 1103, 1080, 1010, 968, 855 and 800 cm\(^{-1}\). Anal. Found: C, 60.96; H, 5.43. Calcd. for C\(_{19}\)H\(_{20}\)O\(_8\): C, 60.63; H, 5.36%.

Cyclohexylidene methylcervicarcin (8) and its rearranged product (10). The benzene solution (70 ml) containing methylcervicarcin (2) (1 g), cyclohexanone (7 ml) and trace of p-toluenesulfuric acid was refluxed under an azeotropic removal of water. 2 first in suspended state became gradually into solution. After refluxing for 4 hr, the two products were detected on thin-layer plate (silicic acid; ethyl acetate-benzene: 3-7); the upper spot was main and the lower one minor. Ratio of the two products inverted while further refluxing, and the lower product became predominantly after 17 hr. Then the reaction solution was washed with M/5 phosphate buffer (pH 7.0), dried (Na\(_2\)SO\(_4\)) and evaporated to dryness. The residue was chromatographed on silicic acid. By eluting the column with 10% ethyl acetate in benzene the two products came out successively. The first eluate was after evaporating the solvents recrystallized from hot ethyl acetate, giving colourless needles of 8; yield 112 mg.

8, mp 177–179°C; \( \nu_{KBr} \) 3500, 2900, 2820, 1721, 1696, 1595, 1578, 1477, 1370, 1290, 1270, 1126, 1105, 1025, 965, 894 and 760 cm\(^{-1}\); \( \delta \) (CDCl\(_3\)) 1.08 (3 H, d, \( J=7.2 \) Hz), 1.42 (3 H, d, \( J=5.4 \) Hz), ca. 1.58 and 1.85 (10 H, broad d, protons of cyclohexylidene ring), 3.07 (1 H, d, \( J=2.0 \) and 5.4 Hz), 3.86 (3 H, s), 3.98 (1 H, d, \( J=2.0 \) Hz), 4.04 (s, OH which disappeared with D\(_2\)O), 4.62 (1 H, d, \( J=3.8 \) Hz), 5.48 (1 H, s), ca. 7.0–7.7 (3 H, m, protons of benzene ring). Anal. Found: C, 63.92; H, 6.10. Calcd. for C\(_{26}\)H\(_{30}\)O\(_9\): C, 64.18; H, 6.22%.

The second eluate containing 10 was after evaporating the solvents recrystallized from ethyl acetate and ligroin; yield 271 mg.

10, mp 137–137°C; \( \nu_{KBr} \) 3460 (broad), 2920, 1765, 1690, 1575, 1472, 1365, 1308, 1280, 1270, 1160, 1125, 1090, 1060 and 920 cm\(^{-1}\); \( \delta \) (CDCl\(_3\)+D\(_2\)O) 1.13 (3H, d, \( J=6.6 \) Hz), 1.55 (3 H, d, \( J=5.0 \) Hz), ca. 1.78 and 1.89 (10 H, broad d, cyclohexylidene protons), 3.85 (1 H, s), 4.05 (1 H, d, \( J=3.0 \) Hz), 4.54 (1 H, d, \( J=3.6 \) Hz), 5.53 (1 H, s) and ca. 7.0–7.8 (3H, m, benzene protons). Anal. Found: C, 64.64; H, 6.36. Calcd. for C\(_{26}\)H\(_{30}\)O\(_9\): C, 64.18; H, 6.22%.

Monoacetate (9) and diacetate (11). Cyclohexylidene derivative (8) (40 mg) was acetylated with acetic anhydride (0.7 ml) and pyridine (2 ml) at room temperature overnight. An excess of the anhydride was decomposed with ethanol and the reaction mixture was evaporated to dryness. The residue was preliminarily purified by washing with \( \text{HCl} \) and \( \text{NaOH} \), and then chromatographed on silicic acid (solvent: 10% ethyl acetate in benzene). Recrystallization from ethyl acetate and ligroin gave colourless needles of monoacetate (9), mp 125–129°C; \( \nu_{KBr} \) ~3500, 2930, 1740, 1718, 1695, 1583, 1450, 1408, 1370, 1250, 1175, 1095, 1025, 930 and 890 cm\(^{-1}\); \( \delta \) (CDCl\(_3\)) 0.92 (3 H, d, \( J=7.2 \) Hz), 1.46 (3 H, d, \( J=5.0 \) Hz), 1.87 (10 H, broad d, cyclohexylidene protons), 2.15 (3 H, s), 3.85 (3 H, s); \( \text{being overlapped with a doublet of 13-H} \), 4.45 (1 H, s), 5.51 (1 H, s), 6.04 (1 H, d, \( J=3.6 \) Hz) and ca. 7.0–7.7 (3 H, m, benzene protons). Anal. Found: C, 64.54; H, 6.36. Calcd. for C\(_{28}\)H\(_{32}\)O\(_{10}\): C, 64.18; H, 6.22%.

Cyclohexylidene rearranged product (10) (63 mg) was acetylated with acetic anhydride (0.8 ml) and pyridine (2 ml) at room temperature overnight. The reaction mixture was purified similarly with the case of monoacetate (chromatography on silicic acid; solvent: 10% ethyl acetate in benzene). Recrystallization from ethyl acetate and ligroin gave colourless needles of monoacetate (9), mp 125–129°C; \( \nu_{KBr} \) ~3500, 2930, 1740, 1718, 1695, 1583, 1450, 1408, 1370, 1250, 1175, 1095, 1025, 930 and 890 cm\(^{-1}\); \( \delta \) (CDCl\(_3\)) 0.92 (3 H, d, \( J=7.2 \) Hz), 1.46 (3 H, d, \( J=5.0 \) Hz), ca. 1.56 and 1.87 (10 H, broad d, cyclohexylidene protons), 2.15 (3 H, s), 3.85 (3 H, s); \( \text{being overlapped with a doublet of 13-H} \), 4.45 (1 H, s), 5.51 (1 H, s), 6.04 (1 H, d, \( J=3.6 \) Hz) and ca. 7.0–7.7 (3 H, m, benzene protons). Anal. Found: C, 64.54; H, 6.36. Calcd. for C\(_{28}\)H\(_{32}\)O\(_{10}\): C, 64.18; H, 6.22%.
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11, mp 230~231°C; ∆KBr 2945, 1778, 1750, 1698, 1600, 1470, 1445, 1365, 1265, 1220, 1115, 1023, 1010 and 922 cm⁻¹; δ (CDCl₃) 0.92 (3 H, d, J=7.5 Hz), 1.48 (3 H, d, J=6.0 Hz), 2.06 (3 H, s), 2.12 (3 H, s), 3.83 (3 H, s), 4.03 (1 H, s), 5.21 (1 H, d, J=9.3 Hz), 5.57 (1 H, s), 5.95 (1 H, d, J=4.8 Hz), ca. 7.0~7.7 (3 H, m, benzene protons). Anal. Found: C, 63.07; H, 5.76. Calcd. for C₃₀H₃₄O₁₁: C, 63.15; H, 5.96%.

Naphthalenic rearranged product (13). The suspension of naphthalenic compound (12) (120 mg) in 1 N sulfuric acid (30 ml) and ethanol (15 ml) was refluxed. 12 was gradually dissolved, becoming into complete solution for 1 hr refluxing. Then the solution was extracted with ethyl acetate, which was after drying (Na₂SO₄) evaporated to dryness. The residue was chromatographed on silicic acid. The product (13) was eluted with 20% ethyl acetate in benzene. Eluate was after evaporating the solvents recrystallized from ethyl acetate and ligroin, giving needles of 13; yield 48 mg.

13, mp 273~277°C; ∆KBr 3540, 2900 (split), 1770, 1595, 1500, 1460, 1372, 1335, 1290, 1260, 1120, 1065, 1035, 993, 933, 803 and 760 cm⁻¹; δ (DMSO-D₆) 0.97 (3 H, d, J=6.8 Hz), 1.36 (3 H, d, J=5.0 Hz), ca. 2.1~3.4 (4 H, m), 3.84 (3 H, s), 3.98 (3 H, s; in which 13-H was hidden), 4.62 (1 H, d, J=10 Hz; which changed into a singlet with D₂O addition), 5.73 (1 H, d, J=10 Hz, 4-OH; which disappeared with D₂O addition), 5.97 (1 H, d, J=6.3 Hz, 13-OH; which disappeared with D₂O addition), 6.9~8.3 (4 H, m, naphthalenic protons).

The tetrol, C₂₁H₂₅O₇, mp 208~210°C, was obtained as an another product from 12 by eluting the chromatographic column with 40% ethyl acetate in benzene; yield 30 mg. It was concluded to be the hydrolyzed product of side chain epoxide to glycol.

Diethylidene methylvicercin (14). The mixed solution of methylvicercin (2) (500 mg), paraldehyde (15 ml) and conc. sulfuric acid (3 drops) was kept standing at room temperature for 20 hr. Ethyl acetate (100 ml) was added to the reaction mixture, which was washed with saturated sodium bicarbonate and water, dried (Na₂SO₄) and evaporated to dryness. The residue was chromatographed on silicic acid. 14 was eluted from the column with 20% ethyl acetate in benzene, and the eluate was after evaporating the solvents recrystallized from ethanol and ligroin, giving colourless needles.

14, mp 264~265°C; ∆KBr ~3000, 2950, 2900, 1736, 1695, 1600, 1585, 1325, 1285, 1153, 1110, 1005 and 920 cm⁻¹; δ (CDCl₃) 1.07 (3 H, d, J=7.2 Hz), 1.29 (3 H, d, J=6.6 Hz), 1.42 (6 H, d, J=5.4 Hz, two Me of diethylidene), 2.01 (1 H, m), 2.94 (1 H, m), 3.90 (3 H, s), 4.57 (1 H, d, J=3.6 Hz), 4.86 (1 H, s), 5.21 (1 H, q, J=5.4 Hz), 5.28 (1 H, q, J=5.4 Hz), 5.63 (1 H, s), and 7.0~7.8 (3 H, m, benzene protons). Anal. Found: C, 61.67; H, 6.15. Calcd. for C₃₄H₂₆O₉·1/2 H₂O: C, 61.66; H, 5.82%.

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