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Structure of an Aromatization Product of C-1027 Chromophore

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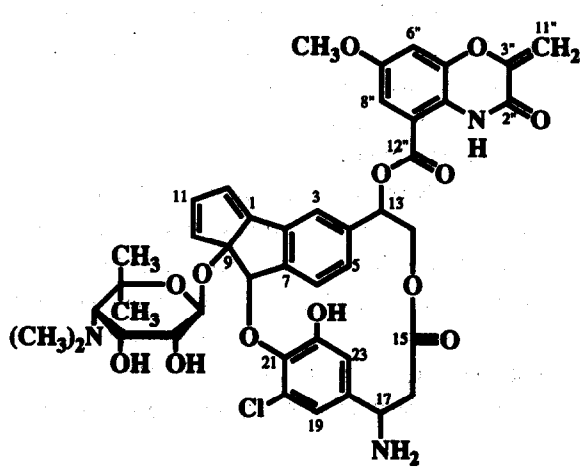
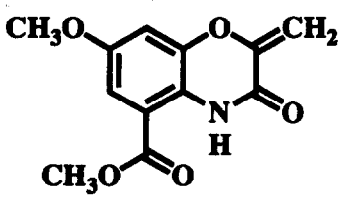
Abstract: **The structure of an aromatization product of C-1027 chromophore was determined by means of chemical degradation and detailed 2D-NMR studies.**

Antibiotic C-1027, a novel antitumor chromoprotein isolated from the broth filtrate of *Streptomyces globisporus* C-1027,[1] shows extremely potent cytotoxicity against KB carcinoma cells (IC_{50} 0.1 ng/ml) *in vitro*[2] and antitumor activity toward tumor-bearing mice *in vivo*.[3] These activities are correlated with the ability of the antibiotic to cause DNA double-strand scission.[2] The antibiotic consists of an apo-protein[4] and a labile chromophore (C-1027-Chr) that is responsible for the biological activity of C-1027. The chromophore is readily separated from its apo-protein by extraction, but the exceeding instability in the protein-free state hampered the structure elucidation. The similar situation has been also observed in the other chromoprotein antibiotics of this family such as neocarzinostatin (NCS),[5] macromomycin,[6] auromomycin (AUR),[7] actinoxanthin,[8] and kedarcidin.[9] Among them, NCS is the only one whose chromophore structure has been elucidated, and very recently, the structural novelty of kedarcidin chromophore has been disclosed by the Bristol-Myers Squibb group. In this communication, we characterize an inactive but more stable reaction product of C-1027-Chr, which was prepared by treatment of C-1027-Chr in methanol as reported previously.[10] It possesses a macrocyclic structure together with oxazolinate and aminosugar moieties as side chains, and will provide us a clue as to the structure of the native C-1027-Chr.



1

Compound 1[11] was isolated as its HCl salt from the inactivated broth filtrate by RP-HPLC (aq. 0.01N HCl-CH_3CN as the mobile phase). Molecular formula of HCl-free 1 was determined by HRFABMS as $C_{43}H_{44}N_3O_{13}Cl$ (Found m/z 846.2643, calcd. for $[M+H]^+$ 846.2640).



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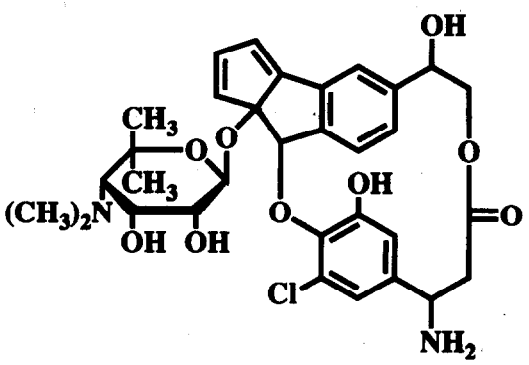

3

Detailed 1D- and 2D-NMR studies of 1, including ^1H-^1H DQF-COSY, ROESY[12] (τ_m=250ms), HMQC,[13] and HMBC[14] (J=5Hz) experiments were performed in DMSO-d_6 solution, and methanolysis (K_2CO_3/CH_3OH) of 1 afforded compounds 2 and 3.[15] Methyl ester 2 proved to be identical with the degradation product of the AUR chromophore reported by Kumada et al.[16] High-field shift of H13, from 5.98 ppm (1) to 4.63 ppm (3) by methanolysis and the HMBC correlation between H13 and the ester carbonyl (164.63 ppm) indicate the ester linkage of this unit to the C13. Three sets of olefinic signals besides those of the oxazolinate unit were observed in 1. The first set of three proton-signals, 6.61 (d, J=2Hz), 6.66 (d, J=5.5Hz), and 6.68 ppm (dd, J=5.5, 2Hz) has coupling constants characteristic to a cyclopentadienyl moiety. The ortho- and meta-couplings of the other two sets indicate the 1,2,4-trisubstituted [6.87 (dd, J=8,1.5Hz), 7.26 (d, J=8Hz), and 7.49 ppm (d, J=1.5Hz)] and 1,2,3,5-tetrasubstituted benzene moieties [6.05 (d, J=2Hz) and 7.16 ppm (d, J=2Hz)]. The ^1H-^{13}C long-range coupling connectivity linked the cyclopentadiene unit with the 1,2,4-trisubstituted benzene, from which the benzodihydropentalene structure was deduced. Moreover, ^1H-^{13}C long-range correlations including those between the phenol proton (8.19 ppm) and aromatic nucleus carbons (C21, C22, and C23) implied that the 1,2,3,5-tetrasubstituted benzene would be a 3'-chloro-5'-hydroxy-β-tyrosine unit (Fig. 1). The correlation of H14 with the ester carbonyl of the β-tyrosine residue (C15, 168.13 ppm) on the HMBC spectrum revealed that the β-tyrosine moiety is linked to C4 of the benzodihydropentalene central core through an ethylene linkage (C13-C14).

Sequential assignment of aminosugar protons starting from the anomeric proton H1' (δ_H 4.53 and δ_C 93.06 ppm) to H4' (3.15 ppm) was readily accomplished. In the HMBC spectrum, the N-dimethyl signal (2.83 ppm) correlated with C4' (69.24 ppm), and moreover two singlet methyl signals (1.48 and 1.50 ppm) did so with C5' (74.89 ppm) and further with C4'. Their vicinal coupling constants ($J_{H1'-H2'}$=8Hz, $J_{H2'-H3'}$=3Hz and $J_{H3'-H4'}$=3Hz) together with the strong NOE correlation peaks among H2', H3', and H4' on the ROESY spectrum established that the structure of the sugar unit should be 4-deoxy-4-dimethylamino-5,5-dimethyl-ribopyranose with an equatorial-glycoside linkage (Fig. 2). The long-range correlation between the anomeric proton (H1') and C9 (99.98ppm) showed that the sugar moiety is attached to C9.

The remaining problem is the connection between C8 and C21. Although the ^{1}H-^{13}C long-range coupling between H8 and C21 could not be observed, probably due to the perpendicularity of the dihedral angle for H-C8-O-C21, the ether linkage is consistent with the molecular formula. Furthermore, the NOE between H12 and the phenol proton in the ROESY spectrum indicates the close proximity of the benzodihydropentalene moiety to the aromatic ring of the β-tyrosine moiety. This strongly supports the macrocyclic structure as well as the cyclophane-like stacked conformation of 1. The trans-stereochemistry of the C9 glycoside and C8 ether bonds was suggested by the NOE between H-8 (6.00 ppm) and the anomeric proton (H1') of the aminosugar.

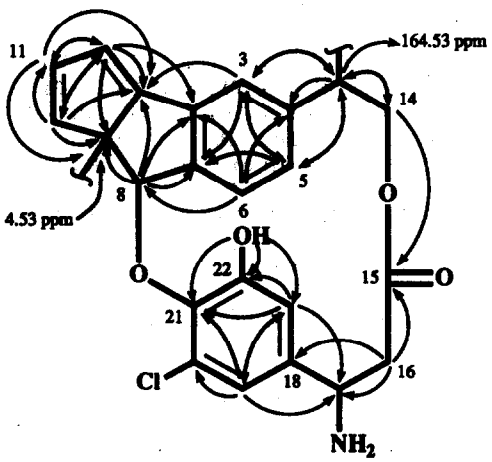

Fig. 1 ^{1}H-^{13}C Long-range Coupling Correlations of the Core Structure of 1.
(Arrows indicate the direction of the correlation as from ^{1}H to ^{13}C.)

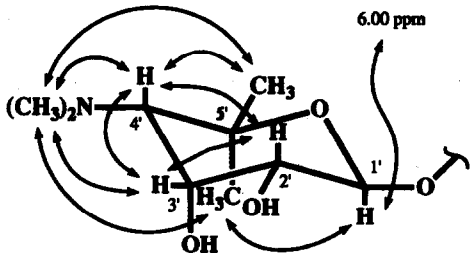

Fig. 2 Relative Stereochemistry and NOE of Aminosugar Residue.

Finally, 2D-INADEQUATE experiment (360 mg/ml in $CDCl_3$, 50°C) was carried out for HCl-free 3 and confirmed unambiguously the ^{13}C-^{13}C connectivity of the core structure as well as the aminosugar moiety. Thus, the novel macrocyclic structure of 1 was established except for the absolute stereochemistry. The benzodihydropentalene moiety of 1 suggested an enediyne structure for the native C-1027-Chr.

Studies on the absolute stereochemistry of 1 are now underway, and the structure of the native chromophore together with its aromatization mechanism will be reported in the following communication.[17]

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11. Bis-HCl salt of 1: colorless amorphous powder: $[\alpha]_D^{20}$-479° (c 0.18, MeOH); IR (KBr) 3400, 2935, 1735, 1685, 1615, 1505, 1445, 1370, 1340, 1255, 1225, 1180, 1150, 1045, 880 and 865 cm^{-1}; UV (MeOH) λ_{max} 285 (62000), 220 (sh), 289 (8100) and 347 nm (13000); ^{1}H-NMR (DMSO-d_6, 400MHz) δ 7.49 (1H, d, J=1.5Hz, 3-H), 6.87 (1H, dd, J=8, 1.5Hz, 5-H), 7.26 (1H, d, J=8Hz, 6-H), 6.00 (1H, s, 8-H), 6.66 (1H, d, J=5.5Hz, 10-H), 6.68 (1H, dd, J=5.5, 2Hz, 11-H), 6.61 (1H, d, J=2Hz, 12-H), 5.98 (1H, dd, J=10.5, 5Hz, 13-H), 4.15 (1H, dd, J=10.5, 10Hz, 14-H), 4.22 (1H, dd, J=10, 5Hz, 14-H), 2.47 (1H, t, J=12.5Hz, 16-H), 3.13 (1H, br.d, J=12.5Hz, 16-H), 4.16 (1H, overlapped, 17-H), 9.04 (3H, br.s, 17-NH_3^+), 7.16 (1H, d, J=2Hz, 19-H), 8.19 (1H, s, 22-OH), 6.05 (1H, d, J=2Hz, 23-H), 4.53 (1H, d, J=8Hz, 1'-H), 2.97 (1H, br.d, J=8Hz, 2'-H), 4.86 (1H, br.s, 2-OH), 4.15 (1H, br.s, 3'-H), 5.55 (1H, d, J=5.5Hz, 3'-OH), 3.15 (1H, br.s, 4'-H), 9.68 (1H, br.s, 4'-NH^+), 2.83 (6H, s, 4'-NMe_2), 1.48 (3H, s, 6'-Meα), 1.50 (3H, s, 6'-Meβ), 10.06 (1H, br.s, 1"-NH), 7.09 (1H, d, J=3Hz, 6"-H), 3.84 (3H, s, 7"-OMe), 7.35 (1H, d, J=3Hz, 8"-H), 5.14 (1H, s, 11"-H), 5.47 (1H, d, J=2Hz, 11"-H); ^{13}C-NMR (DMSO-d_6, 100MHz) δ 150.91 (s, C1), 136.66 (s, C2), 117.02 (d, C3), 139.19 (s, C4), 125.55 (d, C5), 127.14 (d, C6), 146.65 (s, C7), 83.73 (d, C8), 99.98 (s, C9), 134.91 (d, C10), 137.78 (d, C11), 127.41 (d, C12), 72.65 (d, C13), 65.27 (t, C14), 168.13 (s, C15), 41.27 (t, C16), 51.20 (d, C17), 133.59 (s, C18), 115.27 (d, C19), 129.61 (s, C20), 140.01 (s, C21), 151.88 (s, C22), 114.48 (d, C23), 93.06 (d, C1'), 69.79 (d, C2'), 67.37 (d, C3'), 69.24 (d, C4'), 42.53 (q, NMe_2-4'), 44.93 (q, NMe_2-4'), 74.89 (s, C5'), 31.31 (q, C6'α), 21.28 (q, C6'β), 154.47 (s, C2"), 147.31 (s, C3"), 142.14 (s, C5"), 107.43 (d, C6"), 154.47 (s, C7"), 56.00 (q, OMe-7"), 109.26 (d, C8"), 113.63 (s, C9"), 121.01 (s, C10"), 98.98 (t, C11"), 164.63 (s, C12").
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15. Compound 3: HCl salt as a colorless amorphous powder; $C_{32}H_{37}N_2O_9Cl$; FABMS m/z 629 $(M+H)^+$; ^{1}H-NMR (DMSO-d_6, 400MHz) δ 7.41 (1H, d, J=1.5Hz, 3-H), 6.56 (1H, dd, J=7.5, 1.5Hz, 5-H), 7.04 (1H, d, J=7.5Hz, 6-H), 5.88 (1H, s, 8-H), 6.55 (1H, d, J=5.5Hz, 10-H), 6.60 (1H, dd, J=5.5, 2Hz, 11-H), 6.49 (1H, d, J=2Hz, 12-H), 4.63 (1H, dd, J=10.5, 4.5Hz, 13-H), 5.58 (1H, br.s, 13-OH), 3.62 (1H, t, J=10.5Hz, 14-H), 3.82 (1H, dd, J=10.5, 4.5Hz, 14-H), 2.31 (1H, t, J=13Hz, 16-H), 2.97 (1H, dd, J=13, 2Hz, 16-H), 4.06 (1H, m, 17-H), 7.05 (1H, d, J=2Hz, 19-H), 8.11 (1H, br.s, 22-OH), 5.93 (1H, d, J=2Hz, 23-H), 4.43 (1H, d, J=8Hz, 1'-H), 2.92 (1H, dd, J=8, 3Hz, 2'-H), 4.12 (1H, br.s, 3'-H), 3.10 (1H, br.s, 4'-H), 2.77 (3H, s, 4'-NMe), 2.78 (3H, s, 4'-NMe), 1.44 (3H, s, 6'-Meα), 1.42 (3H, s, 6'-Meβ); ^{13}C-NMR (DMSO-d_6, 100MHz) δ 151.14 (s, C1), 136.11 (s, C2), 117.41 (d, C3), 144.59 (s, C4), 125.21 (d, C5), 126.45 (d, C6), 145.64 (s, C7), 83.89 (d, C8), 100.05 (s, C9), 134.89 (d, C10), 137.38 (d, C11), 126.62 (d, C12), 68.87 (d, C13), 68.32 (t, C14), 168.27 (s, C15), 41.42 (t, C16), 51.23 (d, C17), 133.60 (s, C18), 115.07 (d, C19), 129.63 (s, C20), 140.02 (s, C21), 151.91 (s, C22), 114.20 (d, C23), 92.88 (d, C1'), 69.81 (d, C2'), 67.35 (d, C3'), 69.29, (d, C4'), 42.57 (q, NMe-4'), 44.89 (q, NMe-4'), 74.85 (s, C5'), 21.29 (q, C6'α), 31.36 (q, C6'β).
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