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Structure and Cycloaromatization of a Novel Enediyne, C-1027 Chromophore

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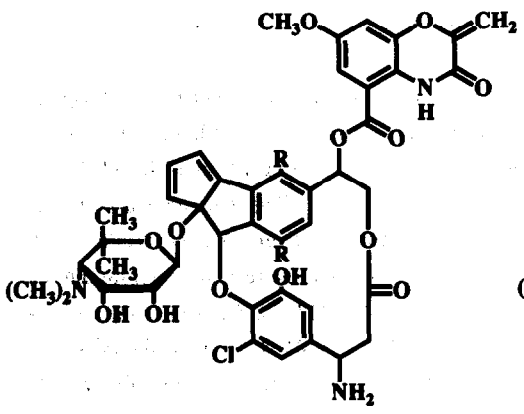
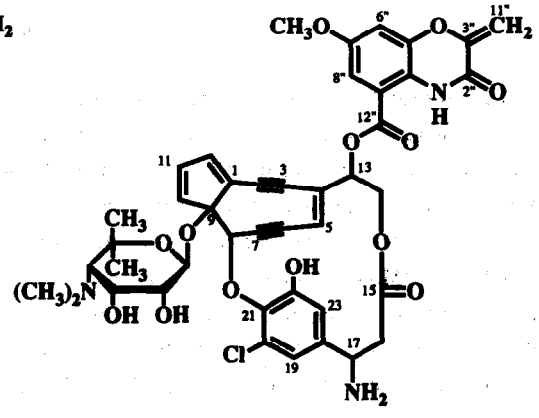
Abstract: The structure and the cycloaromatization mechanism of a novel enediyne, C-1027 chromophore, were elucidated. The C-1027 chromophore has a 9-membered 1,5-diyn-3-ene core structure in the 16-membered macrocyclic ring.

C-1027 Chromophore (C-1027-Chr), an active principle of chromoprotein antibiotic C-1027, has generated much interest because of the extremely potent biological activities of C-1027.[1,2] Since the protein-free C-1027-Chr is too labile to prove the structure only by spectroscopic methods, we first elucidated the structure of the more stable reaction product (**1**) in the preceding communication.[3] Its benzodihydropentalene core structure suggested to us the presence of an enediyne in the native C-1027-Chr. We disclose herein the novel structure of C-1027-Chr (**3**) and the cycloaromatization mechanism leading to product **1**, which would explain its extreme potency in terms of cytotoxicity and ability to cause DNA double-strand scission[2]



1 R=H
2 R=D



3

Highly unstable C-1027-Chr **3** was isolated by RP-HPLC [20mM phosphate buffer (pH 6.86)-CH_3CN as the mobile phase] from an ethyl acetate extract of purified C-1027 complex. Since compound **3** is especially sensitive to light, basic agents, and thiol compounds, all procedures for purification were

performed expeditiously under yellow light to suppress the decomposition. While 3 is relatively more stable in acidic aqueous media than in alkaline solution or in organic solvents, even in acidic solution 3 degrades within one day at room temperature ($t_{1/2}$=10hrs.). Its instability is similar to that of the neocarzinostatin (NCS) chromophore,[4] but C-1027-Chr 3 seems to be ten-fold more labile than the latter.

Compound 3 was obtained as an amorphous pale-yellow solid[5]: $C_{43}H_{42}N_3O_{13}Cl$; FABMS m/z 844 $(M+H)^+$. The molecular weight difference between 3 and reaction product 1 is attributable to the lack of two hydrogen atoms in 3. ^{1}H-NMR spectrum of 3 in acetone-d_6 showed a close similarity with that of HCl-free 1[6] except for the signals due to the central core moiety. The spin-spin coupling connectivities in 3 were confirmed by homo-spin decoupling experiments, and we found that the cyclopentadienyl unit was conserved. Two long-range coupled signals (5.97 and 6.15 ppm, J=2Hz) were observed in the spectrum of 3 instead of the aromatic ABX system (7.43, 7.08 and 7.80 ppm) and a singlet signal (6.22 ppm) in that of 1. The signal at 6.15 ppm was also long-range coupled with H-13. These are consistent with a bicyclo[7.3.0]dodeca-4, 10, 12-trien-2, 6-diyne structure as shown in Fig. 1. Namely, the signals at 5.97 and 6.15 ppm were assigned to H-8 and H-5, respectively. The long-range coupling between H-5 and H-8 through five bonds including an acetylenic bond was also observed in the NCS chromophore.[4]

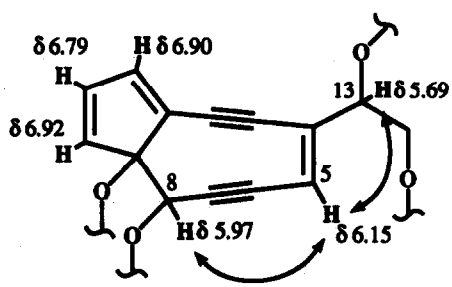


Fig. 1 The Core Structure of C-1027-Chr.
(Arrows indicate ^{1}H-^{1}H long-range couplings)

To confirm the 1,5-diyn-3-ene core structure of 3, we attempted the Masamune-Bergman cycloaromatization reaction.[7] The cycloaromatization reaction of 3 occurred in methanol, ethanol or dioxane. In ethanol, 3 was most efficiently converted into 1: The chromophore fraction of the RP-HPLC eluate was directly mixed with an equal amount of ethanol and heated at 50°C. The reaction was complete within 30 min, and 1 was obtained quantitatively. The reaction was then carried out in ethanol-d_6 (99.5% d) to afford 2 (FABMS m/z 848, $C_{43}D_2H_{42}N_3O_{13}Cl$+H). The presence of H_2O in the reaction medium did not affect the deuterium incorporation. These results demonstrated that the C-1027-Chr 3 abstracts the carbon-bound hydrogens from ethanol, and not from the hydroxyl proton. The ^{1}H-NMR spectra (in DMSO-d_6, as HCl salts) of 1 and 2 are shown in Fig. 2. Both signals of H-3 and H-6 were reduced substantially (deuterium content: 70% at C3 and at C6), and H-5 appeared mainly as a singlet (see arrows in Fig. 2). Therefore, deuteriums were clearly incorporated at C-3 and C-6. These results support the generation of the benzene-1,4-diyl biradical as an intermediate in the Masamune-Bergman cycloaromatization reaction of the enediyne 3 (Scheme 1).

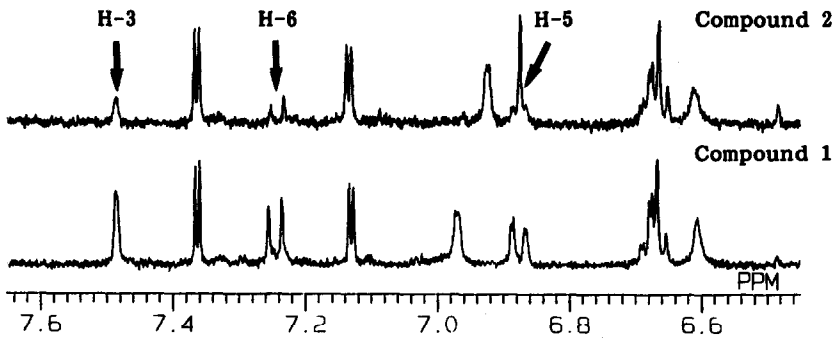
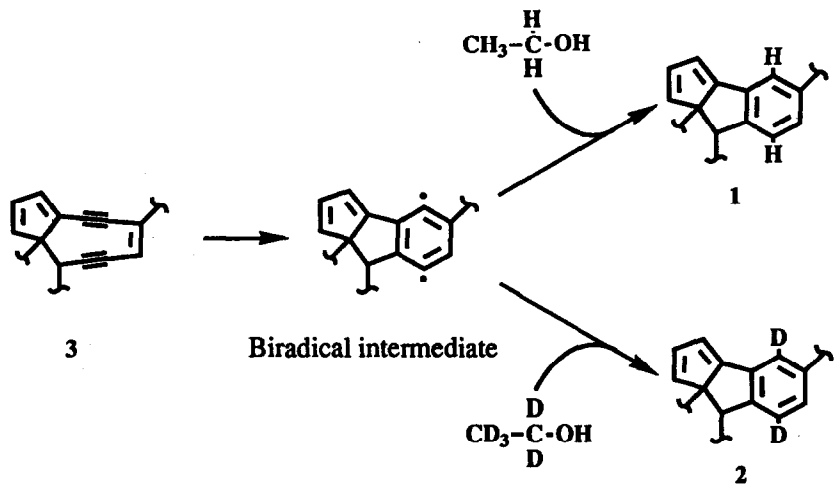


Fig. 2 The ^{1}H-NMR spectra of **1** and **2**. (400 MHz in DMSO-d_6, 30°C, as HCl salts)



Scheme 1. The Cycloaromatization Mechanism of C-1027-Chr.

Thus, C-1027-Chr **3** has been demonstrated to possess a novel, highly strained 9-membered 1,5-diyn-3-ene core structure together with a 16-membered macrocyclic ring, which is different from another 9-membered enediyne, the NCS chromophore, in the enediyne system and the cyclization mechanism.[8] This structure is closely related to that of the kedarcidin chromophore[9] reported very recently by the Bristol-Myers Squibb group. Both have the 9-membered 1,5-diyn-3-ene core, but the C-1027-Chr **3** is the first natural product with 9-membered enediyne core from which the benzene-1,4-diyl biradical generation has been demonstrated.

Whereas the NCS chromophore and 10-membered enediynes, esperamicins,[10] calicheamicins,[11] and dynemicins,[12] have been reported to have some trigger systems in their structure to start the reaction cascade leading to the biradical generation, there seems to be no trigger in C-1027-Chr **3** which undergoes the spontaneous cycloaromatization even at room temperature as the synthetic 9-membered 1,5-diyn-3-enes

do.[13] These facts give rise to a question as how to such a thermally unstable C-1027-Chr **3** is stabilized by the apo-protein. The new enediyne system **3** might have ingenious stabilization interactions and mechanism with the apo-protein upon binding. The macrocyclic structure allows the phenyl ring of the β-tyrosine moiety to be hung over the enediyne system. The β-tyrosine moiety may play an important role for the stabilization of the enediyne system in the protein complex, and the dissociation of **3** from the apo-protein would function as the trigger. There remains another possibility, that a device of covalent bonding[14] between the chromophore and the apo-protein masks the enediyne and **3** would be liberated under certain conditions.

This issue as well as the characteristic sequence specificity in DNA double-strand scission is currently under investigation and will be reported in due course.

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REFERENCES AND NOTES

1. Zhen, Y.; Ming, X.; Yu, B.; Otani, T.; Saito, H.; Yamada, Y. *J. Antibiot.* **1989**, *42*, 1294-1298.
2. Sugimoto, Y.; Otani, T.; Oie, S.; Wierzba, K.; Yamada, Y. *J. Antibiot.* **1990**, *43*, 417-421.
3. Minami, Y.; Yoshida, K.; Azuma, R.; Saeki, M.; Otani, T. *Tetrahedron Lett.*, preceding communication in this issue.
4. Edo, K.; Mizugaki, M.; Koide, Y.; Seto, H.; Furihata, K.; Otake, N.; Ishida, N. *Tetrahedron Lett.* **1985**, *26*, 331-334.
5. C-1027-Chr (**3**): UV (on-line HPLC) λ_{max} 205, 220 (sh), 278, 355 nm; ^{1}H-NMR (acetone-d_6, 400MHz) δ 6.15 (1H,d, J=2Hz, 5-H), 5.97 (1H, d, J=2Hz, 8-H), 6.92 (1H, dd, J=5.5, 1.5Hz, 10-H), 6.79 (1H, dd, J=5.5, 2.5Hz, 11-H), 6.90 (1H, dd, J=2.5, 1.5Hz, 12-H), 5.69 (1H, dd, J=10, 5.5Hz, 13-H), 4.14 (1H, dd, J=10, 5.5Hz, 14-H), 4.17 (1H, t, J=10Hz, 14-H), 2.50 (1H, dd, J=14, 11Hz, 16-H), 2.57 (1H, dd, J=14, 4.5Hz, 16-H), 4.70 (1H, dd, J=11, 4.5Hz, 17-H), 7.00 (1H, d, J=2Hz, 19-H), 6.07 (1H, s, 22-OH), 6.41 (1H, d, J=2Hz, 23-H), 4.30 (1H, d, J=7.5Hz, 1'-H), 3.06 (1H, dd, J=7.5, 2.5Hz, 2'-H), 4.25 (1H, br.t, J=2.5Hz, 3'-H), 2.18 (1H, d, J=2.5Hz, 4'-H), 2.40 (6H, s, 4'-NMe$_2$), 1.31 (3H, s, 6'-Meα), 1.21 (3H, s, 6'-Meβ), 9.99 (1H, br.s, 1"-NH), 6.97 (1H, d, J=3Hz, 6"-H), 3.86 (3H, s, 7"-OMe), 7.34 (1H, d, J=3Hz, 8"-H), 5.07 (1H, d, J=1.5Hz, 11"-H), 5.50 (1H, d, J=1.5Hz, 11"-H).
6. ^{1}H-NMR of **1** (acetone-d_6, 400MHz) δ 7.80 (1H, d, J=1.5Hz, 3-H), 7.08 (1H, dd, J=8, 1.5Hz, 5-H), 7.43 (1H, d, J=8Hz, 6-H), 6.22 (1H, s, 8-H), 6.88 (1H, d, J=5.5Hz, 10-H), 6.82 (1H, dd, J=5.5, 2Hz, 11-H), 6.75 (1H, d, J=2Hz, 12-H), 6.16 (1H, dd, J=10.5, 5.5Hz, 13-H), 4.18 (1H, dd, J=10.5, 9.5Hz, 14-H), 4.23 (1H, dd, J=9.5, 5.5Hz, 14-H), 2.51 (1H, dd, J=14, 11Hz, 16-H), 2.58 (1H, dd, J=14, 4Hz, 16-H), 4.61 (1H, dd, J=11, 4Hz, 17-H), 7.00 (1H, d, J=2Hz, 19-H), 5.38 (1H, br.s, 22-OH), 6.10 (1H, d, J=2Hz, 23-H), 4.67 (1H, d, J=8Hz, 1'-H), 3.05 (1H, dd, J=8, 3Hz, 2'-H), 4.25 (1H, dd, J=3, 2.5Hz, 3'-H), 2.24 (1H, d, J=2.5Hz, 4'-H), 2.48 (6H, s, 4'-NMe$_2$), 1.50 (3H, s, 6'-Meα), 1.31 (3H, s, 6'-Meβ), 10.19 (1H, br.s, 1"-NH), 7.03 (1H, d, J=2.5Hz, 6"-H), 3.97 (3H, s, 7"-OMe), 7.63 (1H, d, J=2.5Hz, 8"-H), 5.12 (1H, d, J=1.5Hz, 11"-H), 5.56 (1H, d, J=1.5Hz, 11"-H).
7. (a) Fujiwara, K.; Hirama, M. *Chemistry Today (Gendai Kagaku)* **1990**, *No. 7*, 14-21; (b) Nicolaou, K. C.; Dai, W. M. *Angew. Chem. Int. Ed. Engl.* **1991**, *30*, 1387-1416.
8. Myers, A. G. *Tetrahedron Lett.* **1987**, *28*, 4493-4496.
9. Leet, J. E.; Schroeder, D. R.; Hofstead, S. J.; Golik, J.; Colson, K. L.; Huang, S.; Klohr, S. E.; Doyle, T. W.; Matson, J. A. *J. Am. Chem. Soc.* **1992**, *114*, 7946-7948.
10. Golik, J.; Dubay, G.; Groenewold, G.; Kawaguchi, H.; Konishi, M.; Krishnan, B.; Ohkuma, H.; Saitoh, K.; Doyle, T. W. *J. Am. Chem. Soc.* **1987**, *109*, 3462-3464.
11. Lee, M. D.; Dunne, T. S.; Chang, C. C.; Ellestad, G. A.; Siegel, M. M.; Morton, G. O.; McGahren, W.J.; Borders, D. B. *J. Am. Chem. Soc.* **1987**, *109*, 3466-3468.
12. Konishi, M.; Ohkuma, H.; Tsuno, T.; Oki, T.; VanDuyne, G. D.; Clardy, J. *J. Am. Chem. Soc.* **1990**, *112*, 3715-3716.
13. Synthetic studies on 9-membered enediynes and the cycloaromatization have been reported. See: (a) Magnus, P.; Pitterna, T. *J. Chem. Soc., Chem. Commun.* **1991**, 541-543; (b) Doi, T.; Takahashi, T. *J. Org. Chem.*, **1991**, *56*, 3465-3467.
14. Actinoxanthin chromophore (structure unknown) was suggested to attach to the hydroxyl group of some serine residue: Khokhlov, A.S.; Reshetov, P. D.; Chupova, L. A.; Cherches, B. Z.; Zhigis, L. S.; Stoyachenko, I. A. *J. Antibiot.* **1976**, *29*, 1026-1034.

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