The Role of the C-1 Triol Group in Bicyclomycin

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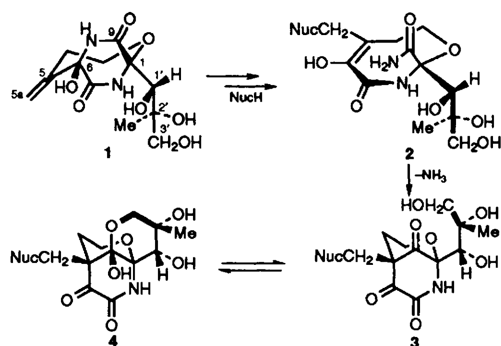
Comparison of the chemical, biochemical, and biological activities of the antibiotic, bicyclomycin, with three C-1 triol modified derivatives demonstrated that all four compounds intercepted thiols but only bicyclomycin effectively inhibited the transcription termination factor rho and possessed significant antimicrobial activity, indicating that the stereochemical and chemical structure of the C-1 triol group played a key role in the drug recognition process.

Bicyclomycin **1** is a structurally distinctive,[1–3] commercial antibiotic whose primary site of drug action in *Escherichia coli* is the essential cellular protein transcription termination factor rho.[4] In 1988, we advanced a novel pathway for the drug activation and protein bonding processes in which C-6 hemiketal ring opening in **1** preceded nucleophilic addition to the newly created enone system (Scheme 1).[5a] Recent attention has focused on the role of the distal C-1 triol group in bicyclomycin bonding processes. There is evidence that hydrogen bond interactions between this unit and the C-9 carbonyl group can assist hemiketal ring opening in bicyclomycin and in bicyclomycin analogues at moderate[5c,6] and high[7] pH values. The role of this unit in biological processes, however, need not be restricted to drug activation. The C-1 triol group may be instrumental in rho's recognition of the antibiotic.[4,8] Herein, we provide findings to support this hypothesis and the essential nature of the C-1 triol unit for drug function.

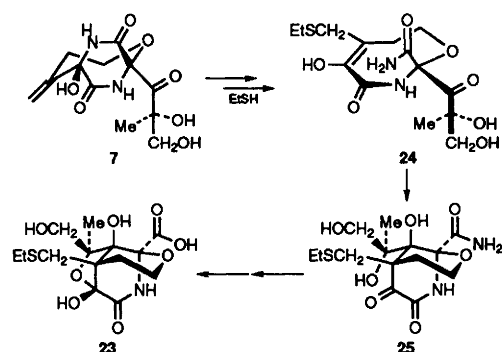
Our study involved the bicyclomycin derivatives **5** (1′*R*, 2′*S*) and **6** (1′*S*, 2′*R*), in which the stereochemical configuration at C-1′ and C-2′, respectively in **1** (1′*S*, 2′*S*) had been reversed, and the C-1′ ketone analogue **7** (2′*S*), in which the stereogenic centre at C-1′ in **1** had been removed. Synthesis of the C-1′ epimer **5** and the C-1′ ketone **7** analogues required the initial ketalization of bicyclomycin to give acetonide **8**.[9] Swern oxidation [*i*, DMSO, $(CF_3CO)_2O$, −70 °C; *ii*, NEt_3][10] of **8** produced **9**,†[11] which was then deprotected to give **7**.‡ $NaBH_4$ reduction of **9** regenerated **8**. No evidence of the corresponding stereoisomer **10** was observed (^1H NMR analysis). Comparable results were obtained using $LiAlH_4$, $NaAlH_2(OCH_2CH_2OMe)_2$, lithium 9-borabicyclo[3.3.1]nonanyl hydride and $Bu^i{}_2AlH$. We synthesized the alternative stereoisomer **10** under Luche conditions with $NaBH_4$–$CeCl_3$.[12] This method rendered a 55:45 mixture of **10** and **8**. Chromatographic separation of **10**[11] followed by deprotection of the acetonide linkage produced **5**.§ Synthesis of the C-2′ hydroxy isomer **6** proceeded *via* C-3′ mesylate **11**.[13] Conversion of **11** to epoxide **12**[13] followed by treatment with dilute methanolic sulfuric acid gave the novel (9-O–C-2′) cyclized adduct **13**,¶ in which epoxide ring opening occurred with stereochemical inversion. Hydrolysis [dilute H_2SO_4 in THF–H_2O (3:1)] of **13** in the final step yielded **6**.‖ The C-2′ stereochemical configuration was confirmed by treatment of **6** with 2,2-dimethoxypropane and toluene-*p*-sulfonic acid to give acetonides **14** and **15**, and then determining the X-ray crystal structure of **15**.[11]



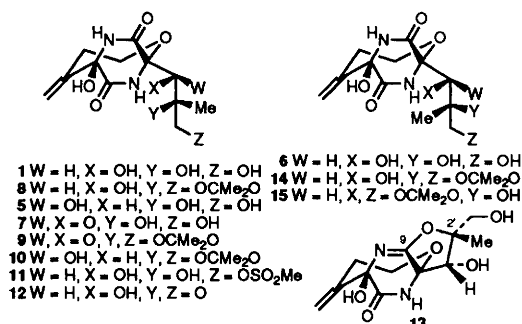
Scheme 1

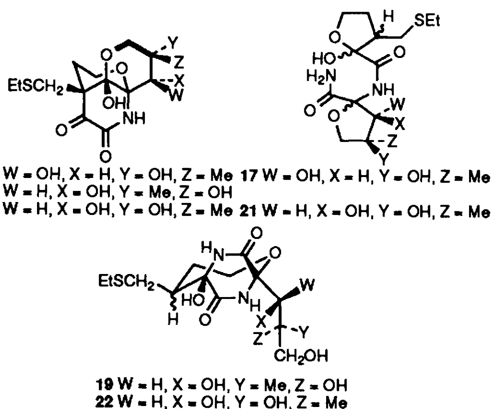
The chemical reactivities of **5–7** with ethanethiol were evaluated to determine the effect of C-1 triol modification on C-5–C-5a functionalization. Ethanethiol was chosen as the nucleophile based on the early projections of Someya, Iseki and Tanaka that protein sulfhydryl groups were the likely species intercepted by bicyclomycin.[14] The specific site of bicyclomycin covalent attachment to rho has not yet been determined. Addition of ethanethiol to a THF–H_2O (3:1) solution ('pH' 8.1) containing **5** gave piperidinedione **16** along with the ring-opened adduct **17**. Correspondingly, **6** at 'pH' 8.6 yielded **18** and **19**. The structures of **16** and **18** and of **17** and **19** differed only in the stereochemistry at the C-1′ or C-2′ sites, respectively, from the ethanethiol addition products **20** (**4**), **21** and **22** previously obtained from **1**.[5a,c] Treatment of C-1′ ketone **7** with ethanethiol ('pH' 8.2) furnished only **23**.¶ We suspect that formation of **23** proceeded by a pathway similar to the one described in Scheme 1 for **4** (**20**), except that enol **24** underwent an intramolecular aldol condensation at the C-1′ carbonyl site instead of a Claisen condensation at the C-9 amide centre (Scheme 2).

The reactivities of **5–7** *vs.* **1** towards ethanethiol were determined by competition experiments. Pairing equimolar mixtures of bicyclomycin with the C-1 triol-modified derivatives **5–7** and treating the pairs with ethanethiol (4–6 equiv.) [0.1 mol dm^{-3} tris·HCl, THF–H_2O (3:1), 'pH' 8.1–8.6, room temp.] demonstrated that consumption of **5–7** proceeded at rates comparable to bicyclomycin (*i.e.* **5** vs. **1**, 2.5:1; **6** *vs.* **1**, 0.7:1; **7** *vs.* **1**, 5:1). The rapidity of the ethanethiol-mediated consumption of **7** was surprising. Compound **7** lacked a C-1′ hydroxy group.** Studies have shown that the C-1′ hydroxy group can promote drug activation by forming an intramo-



Scheme 2





lecular hydrogen bond.[5c,6,7] We have attributed the reactivity of **7** *vs.* **1** with ethanethiol to the multistep nature of these transformations. We suspect that the decrease in the equilibrium constant for hemiketal ring opening of **7** *vs.* **1** is offset by the enhanced rate of the aldol *vs.* the Claisen condensation reactions required for piperidine ring formation.

The ease with which the exomethylene group in compounds **5–7** was modified by ethanethiol raised the possibility that these three bicyclomycin derivatives might display antibiotic activity (Scheme 1). Accordingly, we first evaluated the ability of **5–7** to react with the bicyclomycin target, rho, using the rho-dependent poly(C)-stimulated ATPase assay.[15] Compounds **5–7** did not effectively inhibit rho-dependent hydrolysis of ATP at the concentration levels (400-μmol dm^{-3}) observed to block ATPase activity by bicyclomycin [% inhibition of ATPase activity: **1** (95%), **5** (35%), **6** (25%), **7** (0%)]. When we used a filter disc microbiological assay,[16] none of the three compounds exhibited noticeable antibiotic activity against *E. coli* W3350 cells, while **1** produced a significant zone of inhibited bacterial cell growth (minimal inhibitory concentration: **1** (250 μg cm^{-3}), **5** (>1200 μg cm^{-3}), **6** (>1200 μg cm^{-3}), **7** (>1200 μg cm^{-3}).

The structure–activity results for compounds **1** and **5–7** demonstrated that reactivity towards thiol addition in bicyclomycin derivatives was not a predictor of either biochemical or biological activity.[8] Moreover, the inability of **5–7** to appreciably inhibit rho-dependent poly(C)-stimulated ATPase activity provided evidence that key interactions existed between the C-1 triol group in **1** and the bicyclomycin binding site in rho that are necessary for drug utilization. These interactions along with the catalytic roles of the C-1 triol group in the activation of the antibiotic[5c,6,7] emphasized the fundamental importance of this unit for bicyclomycin function.

We thank Dr James D. Korp for the X-ray crystallographic analyses of **9, 10, 13, 15** and **23** (tetra-*n*-butylammonium salt), Dr William R. Widger, H. G. Park and M. Eichberg for their help in determining the activities of **1** and **5–7** in the rho dependent poly(C)-mediated ATPase and microbiological assays, and Dr Simon Gaskell for the many high-resolution MS studies. We also thank Dr M. Kawamura and the Fujisawa Pharmaceutical Co., Ltd., Japan, for providing bicyclomycin, and Dr T. Platt (University of Rochester) for the over-producing strain of rho. This work was supported by NIH grant (GM37934) and the Robert A. Welch Foundation (E-607).

Received, 7th February 1994; Com. 4/00741G

Footnotes

† All new compounds were fully characterized by ^1H NMR, ^{13}C NMR, MS and high resolution MS.

‡ Compound **7**: R_f 0.39 (20% MeOH–$CHCl_3$); ^1H NMR (CD_3OD) δ 1.35 (s, 3 H), 2.62–2.69 (m, 2), 3.45 (d, *J* 10.5 Hz, 1 H), 3.74 (d, *J* 10.5 Hz, 1 H), 3.80–3.90 (m, 1 H), 4.02–4.12 (m, 1 H), 5.16 (s, 1 H), 5.58 (s, 1 H).

§ Compound **5**: R_f 0.28 (20% MeOH–$CHCl_3$); ^1H NMR (CD_3OD) δ 1.21 (s, 3 H), 2.58 (dd, *J* 7.0, 16.2 Hz, 1 H), 2.65 (dd, *J* 9.0, 16.2 Hz, 1 H), 3.41 (d, *J* 10.9 Hz, 1 H), 3.56 (d, *J* 10.9 Hz, 1 H), 3.80 (dd, *J* 9.0, 13.1 Hz, 1 H), 3.97 (dd, *J* 7.0, 13.1 Hz, 1 H), 4.24 (s, 1 H), 5.13 (s, 1 H), 5.56 (s, 1 H, 5a-H*H*).

¶ *Crystal data*: $C_{12}H_{16}N_2O_6 \cdot 1/2H_2O$, space group $P2_1$, $a = 10.228(3)$, $b = 12.349(4)$, $c = 11.564(2)$ Å, $\beta = 112.00(2)°$, $V = 1354$ Å^3, $Z = 4$, $D_c = 1.44$ g cm^{-3}, μ (Mo-Kα) = 1.10 cm^{-1}, 3255 unique reflections; 2881 reflections with $I > 3\sigma(I)$: $R = 0.042$, $R_w = 0.033$.

Crystal data for tetra-*n*-butylammonium salt: $C_{16}H_{36}N^+ \cdot C_{14}H_{20}NO_8S^-$, space group $P2_12_12_1$, $a = 10.803(2)$, $b = 13.065(2)$, $c = 23.497(5)$ Å; $V = 3316$ Å^3, $Z = 4$, $D_c = 1.21$ g cm^{-3}, μ(Mo-Kα) = 1.38 cm^{-1}, 3672 unique reflections; 2722 reflections with $I > 3\sigma(I)$: $R = 0.039$, $R_w = 0.030$. Atomic coordinates, bond lengths and angles, and thermal parameters have been deposited at the Cambridge Crystallographic Data Centre. See Information for Authors, Issue No. 1.

‖ Compound **6**: R_f 0.39 (20% MeOH–$CHCl_3$); ^1H NMR (CD_3OD) δ 1.32 (s, 3 H), 2.58–2.65 (m, 2 H), 3.32 (d, *J* 10.5 Hz, 1 H), 3.65 (d, *J* 10.5 Hz, 1 H), 3.78–3.96 (m, 2 H), 4.19 (s, 1 H), 5.14 (s, 1 H), 5.56 (s, 1H).

** No evidence was obtained that the C-1′ carbonyl group was hydrated in either D_2O or [$^2H_8$]THF–D_2O (3 : 1) (^{13}C NMR analysis).

References

1 T. Miyoshi, N. Miyairi, H. Aoki, M. Kohsaka, H. Sakai and H. Imanaka, *J. Antibiot.*, 1972, **25**, 569.
2 S. Myamura, N. Ogasawara, H. Otsuka, S. Niwayama, H. Tanaka, T. Take, T. Uchiyama, H. Ochiai, K. Abe, K. Koizumi, K. Asao, K. Matsuki and T. Hoshino, *J. Antibiot.*, 1972, **25**, 610.
3 R. M. Williams and C. A. Durham, *Chem. Rev.*, 1988, **88**, 511.
4 A. Zwiefka, H. Kohn and W. R. Widger, *Biochemistry*, 1993, **32**, 3564.
5 (*a*) S. Abuzar and H. Kohn, *J. Am. Chem. Soc.*, 1988, **110**, 4089; (*b*) 1989, **111**, 4895; (*c*) 1990, **112**, 3114; (*d*) S. Abuzar and H. Kohn, *J. Org. Chem.*, 1989, **54**, 4000.
6 M. A. Vela and H. Kohn, *J. Org. Chem.*, 1992, **57**, 5223.
7 (*a*) R. M. Williams, K. Tomizawa, R. W. Armstrong and J.-S. Dung, *J. Am. Chem. Soc.*, 1985, **107**, 6419; (*b*) R. M. Williams, K. Tomizawa, R. W. Armstrong and J.-S. Dung, *J. Am. Chem. Soc.*, 1987, **109**, 4028.
8 R. M. Williams, R. W. Armstrong and J.-S. Dung, *J. Med. Chem.*, 1985, **28**, 733.
9 T. Kamiya, S. Maeno and Y. Kitaura, Belg. Pat., 847,475.
10 K. Omura, A. K. Sharma and D. Swern, *J. Org. Chem.*, 1976, **41**, 957.
11 The X-ray crystallographic data for this compound will be published elsewhere.
12 (*a*) J.-L. Luche, L. Rodriguez-Hahn and P. Crabbé, *J. Chem. Soc., Chem. Commun.*, 1978, 601; (*b*) K. Li, L. G. Hamann and M. Koreeda, *Tetrahedron Lett.*, 1992, **33**, 6569.
13 B. W. Müller, O. Zak, W. Kump, W. Tosch and O. Wacker, *J. Antibiot.*, 1979, **32**, 689.
14 A. Someya, M. Iseki and N. Tanaka, *J. Antibiot.*, 1979, **32**, 402.
15 J. A. Sharp, J. L. Galloway and T. Platt, *J. Biol. Chem.*, 1983, **258**, 3482.
16 H. M. Ericsson and J. C. Sherris, *Acta Pathol. Microbiol. Scand.*, 1971, Suppl. 217, 1.