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Reductive Thioalkylation of a Kalafungin Analogue

Margaret A. Brimble* and Michael R. Nairn

School of Chemistry, University of Sydney, NSW 2006, Australia

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Abstract: Reduction of pyranonaphthoquinone **3** with sodium dithionite in the presence of the sulfur nucleophiles thiocresol, benzylmercaptan and butanethiol afforded the thioalkylated products **4,5**; **6,7**; and **8,9** respectively. The isolation of these products provides the first example of the ability of pyranonaphthoquinone antibiotics such as kalafungin **1** to undergo reductive thioalkylation at the C-4 position.

One of our synthetic programs has been directed towards the synthesis of the pyranonaphthoquinone antibiotics, members of which include kalafungin **1**,[1] nanaomycin A,[2] frenolicin,[3] medermycin[4] and griseusin A.[5] Apart from their already documented activity[6] against Gram-positive bacteria, fungi and mycoplasmas, it has been proposed that *in vivo* reduction of pyranonaphthoquinones such as kalafungin **1** causes a transformation to an active hydroquinone form **2**, which upon molecular rearrangement may function as an alkylating agent or a *bis*-alkylating agent (Scheme 1).[7,8] If the nucleophile involved were the nitrogenous base of a DNA molecule this proposed mechanism of action resembles that of the anticancer agent mitomycin C.[8]

Despite the obvious potential of pyranonaphthoquinones to act as bioreductive alkylating agents, it is somewhat surprising that no investigation of their ability to act in this manner has been reported. We therefore herein report our studies on the reductive thioalkylation of an analogue of kalafungin **3** which provides the first evidence for isolation of thioalkylation products analogous to that postulated by Moore.[7,8]

The concept of bioreductive activation of a quinone functionality has been well demonstrated using mitomycin C and analogues,[9,10] anthracycline antitumour drugs[11] and model quinone methides.[12] We therefore examined the reduction of kalafungin analogue **3**[13] in the presence of various nucleophiles with several reducing agents which had been successfully used in these related studies.

Sodium dithionite,[9,10] 4-methoxyphenylhydrazine,[14] sodium borohydride,[15] PtO_2/H_2,[16] and chromium(II) perchlorate[17] were examined as reducing agents in the presence of imidazole, thiocresol, dithiothreitol and aniline as nucleophiles. Despite extensive experimentation success was only realised using the sulfur based nucleophile, thiocresol, and the optimum reducing agent proved to be sodium dithionite in 1:1 MeOH/THF buffered at pH 7.4 with Trizma. The lack of success in reacting pyranonaphthoquinone **3** with nucleophiles other than thiocresol was reminescent of recent work[12] which focused on anthracycline systems. In this case sulfur based adducts afforded stable adducts while oxygen and nitrogen based nucleophiles were thought to form unstable adducts due to the reversibility of the addition.

Treatment of pyranonaphthoquinone **3** with sodium dithionite in the presence of thiocresol followed by the addition of diazomethane to the crude reaction mixture afforded a mixture of the isomeric thioalkylated products **4 and 5** in 3.1:1 ratio as determined by ^{1}H NMR and HPLC (Scheme 2). The two isomers were

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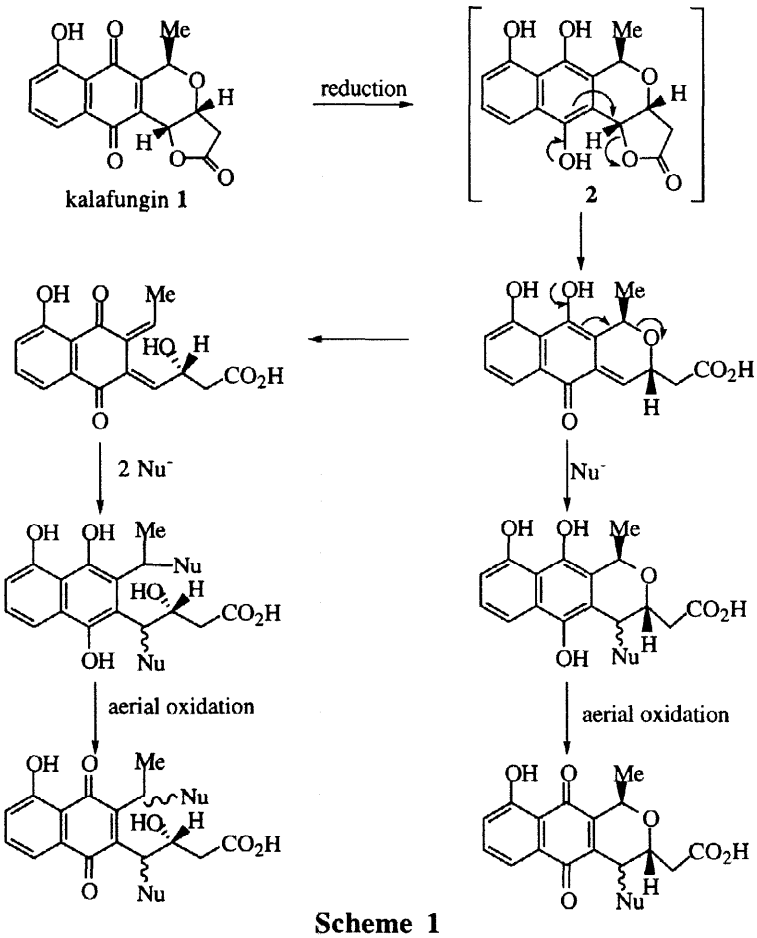
carefully separated by preparative HPLC (with substantial loss of material) in order to carry out full characterisation.[18] The minor isomer **5**, however, was found to convert to the major isomer **4** upon standing.



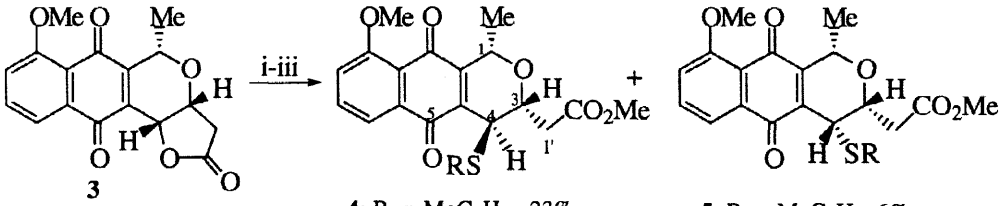
Scheme 1

The stereochemistry of the thiol adducts **4,5** was determined by the magnitude of the vicinal coupling between H-3 and H-4 (Figure). Given that the methyl group at C-1 and the ester side chain at C-3 adopt pseudoequatorial positions on the flattened six membered ring, the large diaxial coupling constant, $J_{3,4}$ = 13.9 Hz, observed for the major isomer **4**, established that the thiol group adopted a pseudoequatorial position. In the minor isomer **5**, $J_{3,4}$ = 2.0 Hz established that the thiol group adopted a pseudoaxial position. These assignments are consistent with preferential attack of the thiol from the top face of the molecule thereby avoiding unfavourable steric hindrance from the ester side chain at C-3.

Given the success realised using thiocresol as a nucleophile, benzyl mercaptan and butyl mercaptan were also employed and the analogous adducts isolated (**6,7** and **8,9** respectively). The benzyl mercaptan adducts **6,7** required separation by preparative HPLC, however, the butyl mercaptan adducts **8,9** were separable by flash chromatography. More complex thiols such as *N*-acetyl cysteine and potassium ethyl xanthate were also used, however, the adducts proved to be extremely difficult to isolate. Carbon (sodium diethylmalonate)[13] and oxygen (ethanol and ethoxide)[13] based nucleophiles were also employed, however, no isolable material was recovered from these experiments.

Reduction of pyranonaphthoquinone **3** with 4-methoxyphenylhydrazine[14], sodium borohydride[15] and PtO_2/H_2[16] as reducing agents led to rapid decolourisation of **3** and formation of a more polar product

(presumably the hydroquinone) as observed by tlc. Subsequent addition of thiocresol led to formation of the thioalkylation product **4,5** albeit in lower yield than that obtained using dithionite as the reducing agent. Whilst these latter experiments supported the hypothesis that reduction to a hydroquinone preceded the thioalkylation step we were concerned that the thiol may be acting as the reducing agent. Thus when pyranaonaphthoquinone **3** was treated with thiocresol in the absence of dithionite thioalkylation products **4,5** were formed together with di-*p*-tolyl disulfide. This latter observation suggests that thioalkylation may in fact be proceeding *via* a semiquinone radical - thiyl radical coupling.



3

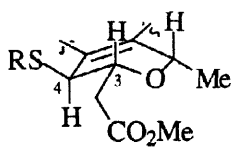
4: R=*p*-MeC_6H_4, 23%
6: R=$C_6H_5CH_2$, 24%
8: R=$Me(CH_2)_3$, 52%

5: R=*p*-MeC_6H_4, 6%
7: R=$C_6H_5CH_2$, 9%
9: R=$Me(CH_2)_3$, 11%

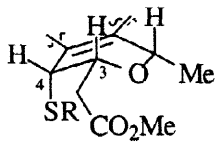
Reagents and Conditions: (i) sodium dithionite, 1:1 THF:MeOH, Trizma, Ar, then RSH (4.0 equiv RT, 4h.; (ii) oxidation (air); (iii) Et_2O, CH_2N_2.

Scheme 2

In summary, the isolation of thioalkylation products from the reaction of pyranonaphthoquinone **3** with thiols after reduction with sodium dithionite and other reducing agents, provides the first experimental evidence that pyranonaphthoquinones such as **3** can undergo reductive thioalkylation at C-4. Whilst the precise mechanism by which thioalkylation proceeds remains open for discussion, naturally pyranonaphthoquinone antibiotics such as kalafungin **1** may undergo similar reductive thioalkylations mediated by an enzyme in a similar manner to the mechanism postulated by Moore.[7,8] The work reported herein has focussed on the use of thiols as nucleophiles, however, this is attributed to the difficulties associated with the isolation of the alkylation products using alternative nucleophiles.



$J_{3,4}$ = 13.9 Hz
4



$J_{3,4}$ = 2.0 Hz
5

Figure

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REFERENCES AND NOTES

1. Hoeksama, H.; Kruger, W. C. *J. Antibiot.*, **1976**, 29, 704.
2. Tanaka, H.; Koyama, Y.; Awaya, J.; Marumo, H.; Oiwa, R.; Katagiri, M.; Nagai, T.; Omura, S. *J. Antibiot.*, **1975**, 27, 860.
3. Iwai, Y.; Kora, A.; Takahashi, Y.; Awaya, T.; Masuma, R.; Oiwa, R.; Omura, S., *J. Antibiot.*, **1978**, 31, 959.
4. Karwowski, J. P.; Jackson, M.; Theriault, R. J.; Prokop, J. K.; Maus, M. L. *J. Antibiot.*, **1988**, 41, 1205.
5. (a) Tsuji, N.; Kobayashi, M.; Wakisaka, Y.; Kawamura, Y.; Mayama, M.; Matsumoto, K. *J. Antibiot.*, **1976**, 29, 7. (b) Tsuji, N.; Kobayashi, M.; Terui, Y.; Tori, K. *Tetrahedron*, **1976**, 32, 2207.
6. (a) Johnson, L. E.; Dietz, A. *Appl. Microbiol.*, **1968**, 16, 1815. (b).Tatsuta, K.; Akimoto, K.; Annaka, M.; Ohno, Y.; Kinoshita, M. *Bull. Chem. Soc. Jpn.*, **1985**, 58, 1699.
7. Moore, H.W. *Science*, **1977**, 197, 527.
8. Moore, H.W.; Czerniak, R. *Med. Res. Rev.*, **1981**, 1, 249.
9. Schiltz, P.; Kohn, H. *J. Am. Chem. Soc.*, **1993**, 115, 10497.
10. Schiltz, P.; Kohn, H. *J. Am. Chem. Soc.*, **1993**, 115, 10510.
11. Taatjes, D. J.; Gaudiano, G.; Resing, K.; Koch, T. H. *J. Med. Chem.*, **1997**, 40, 1276.
12. Angle, S. R.; Rainier, J. D.; Woytowicz, C. *J. Org. Chem.*, **1997**, 62, 5884 and references therein.
13. Brimble, M.A.; Stuart, S.J. *J. Chem. Soc., Perkin Trans I*, **1990**, 880.
14. (a) Han, I.; Russell, D.J.; Kohn, H. *J. Org. Chem.*, **1992**, 57, 1799. (b) Russell, D.J.; Kohn, H. *Heterocycles*, **1990**, 31, 223.
15. Li., V.-S.; Kohn, H. *J. Am. Chem. Soc.*, **1993**, 113, 275.
16. Subramaniam, S.; Kohn, H. *J. Am. Chem. Soc.*, **1993**, 115, 10519.
17. (a) Hong, Y.P.; Kohn, H. *J. Am. Chem. Soc.*, **1991**, 113, 4634. (b) Hong, Y.P.; Kohn, H. *J. Org. Chem.*, **1991**, 56, 6479.
18. **4** was isolated as a yellow solid (8.5 mg, 23%) after purification by HPLC (R_t 64.4 min.), m.p. 156-158°C (Found: MH^+, 453.13671. $C_{25}H_{25}O_6S$ requires MH^+, 453.13718); υ_{max} (CH_2Cl_2)/cm^{-1} 1740s (C=O, ester), 1662s,1652s (C=O, quinone), 1586s (C=C) and 1266s (C-O); δ_H (400 MHz; $CDCl_3$) 1.44 (3H, d, *J* 6.7, CH*Me*), 2.28 (3H, s, C_6H_4*Me*), 2.62 (1H, dd, J_{gem} 15.6 and $J_{1'A,3}$ 8.3, C*H*^{A}CO_2Me), 3.09 (1H, dd, J_{gem} 15.6 and $J_{1'B,3}$ 3.0, C*H*^{B}CO_2Mc), 3.72 (3H, s, CO_2Me), 3.99-4.07 (1H, m, 4-H), 4.00 (3H, s, OMe), 4.04 (1H, ddd, $J_{3,4}$ 13.9, $J_{3,1'A}$ 8.3 and $J_{3,1'B}$ 3.0, 3-H), 4.46 (1H, qd, $J_{1,Me}$ 6.7 and $J_{1,4}$ 2.2, *CH*Me), 7.03 (2H, d, *J* 7.9, C_6H_4Me), 7.26-7.31 (3H, m, C_6H_4Me and 6-H or 8-H), 7.67-7.71 (1H, m, 7-H) and 7.81 (1H, dd, *J* 7.6 and *J* 1.0, 8-H or 6-H; *m/z* (LSIMS, mnba) 453 (MH^+, 63), 421 (MH-MeOH, 13), 379 (MH-MeOH-CH_2CO, 9), 330 (MH-MeC_6H_4S, 100), 292 (23), 273 (45), 242 (36) and 227 ($C_{14}H_{11}O_3$, 37). **5** was isolated as a yellow solid (2.2 mg, 6%) after purification by HPLC (R_t 67.8 min.), m.p. 58-60°C (Found: MH^+, 453.13703. $C_{25}H_{25}O_6S$ requires MH^+, 453.13718); υ_{max} (CH_2Cl_2)/cm^{-1} 1735s (C=O, ester), 1663s,1654s (C=O, quinone), 1586s (C=C) and 1267s (C-O); δ_H (200 MHz; $CDCl_3$) 1.43 (3H, d, *J* 6.7, CH*Me*), 2.32 (3H, s, C_6H_4*Me*), 2.85 (1H, dd, J_{gem} 16.7 and $J_{1'A,3}$ 6.7, C*H*^{A}CO_2Me), 2.97 (1H, dd, J_{gem} 16.7 and $J_{1'B,3}$ 6.7, C*H*^{B}CO_2Me), 3.53 (3H, s, CO_2Me), 4.00 (3H, s, OMe), 4.14 (1H, ddd, $J_{3,1'A}$ 6.7, $J_{3,1'B}$ 6.7 and $J_{3,4}$ 2.0, 3-H), 4.39 (1H, dd, $J_{4,3}$ 2.0 and $J_{4,1}$ 2.0, 4-H), 4.87 (1H, qd, $J_{1,Me}$ 6.7 and $J_{1,4}$ 2.0, *CH*Me), 7.11 (2H, d, *J* 7.9, C_6H_4Me), 7.21-7.35 (2H, m, C_6H_4Me), 7.54-7.81 (3H, m, 6-H, 7-H and 8-H); *m/z* (LSIMS, mnba) 453 (MH^+, 84), 379 (MH-MeOH-CH_2CO, 6), 330 (MH-MeC_6H_4S, 100), 273 (27), 255 ($C_{15}H_{11}O_4$, 25) and 227 ($C_{14}H_{11}O_3$, 32).