Tetrahedron Letters, Vol. 33, No. 41, pp. 6089-6092, 1992
Printed in Great Britain

0040-4039/92 $5.00 + .00
Pergamon Press Ltd

pH-Dependent Stability and Reactivity of a Thiol-Quinone Methide Adduct

Steven R. Angle* and Wenjin Yang
Department of Chemistry, University of California
Riverside, California 92521

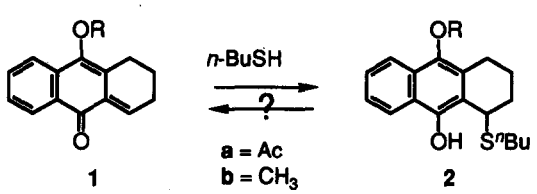
Key words: Quinone methides; anthracycline antitumor antibiotics; pH dependent

Abstract: 10-Acetyl-1-(butanethio)-1,2,3,4-tetrahydro-9,10-anthracenediol **2a**, a quinone methide-butanethiol adduct has been prepared in 63% yield. The pH-dependent stability and reaction with 2'-deoxyadenosine showed that the nucleoside can be exchanged for the thiol at pH 7.

Quinone methides have been proposed to be potentially important intermediates in the chemistry and mode of action of quinonoid antitumor compounds.[1] For example, the anthracycline antitumor antibiotics are thought to derive at least some of their biological activity via *in vivo* quinone methide formation followed by alkylation of a critical biomolecule such as DNA.[1a] Due to the instability of the quinone methides derived from the anthracyclines, a thorough investigation of the chemistry of these intermediates has not been possible.[2-6] The work presented here is part of our continuing effort [7] to study the chemistry of simple quinone methides. We hope to obtain a better understanding of these reactive intermediates and use the results as calibration for what might be expected from quinone methides derived from the anthracyclines. In addition the study might lead to methods for the selective modification of DNA using quinone methides.

In the aqueous intracellular environment, the thiol group is believed to be one of the most important and strongest nucleophiles. If a quinone methide were to be an alkylating agent *in vivo*, one might expect them to be rendered inactive by nucleophilic addition of a thiol (*i.e.* **1** to **2**, equation 1). The study presented here examines the pH dependent stability and reactivity of thiol-quinone methide adduct **2a**.

(equation 1)

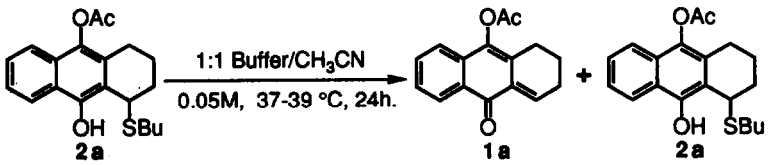


1 **2**

We have previously reported the synthesis of quinone methides **1a** and **1b**.[7] We chose to study butanethiol-acetyloxy quinone methide adduct **2a**, due to its stability and solubility. Accordingly, butanethiol (1.0 equiv) was added to a $CDCl_3$ solution of quinone methide **1a**[7] (0.33 M, 2 min, ^1H NMR monitoring) to afford **2a** in 63 % yield after flash chromatography. [8]

Adduct **2a** was stable, both neat and in solution ($CDCl_3$, CH_3CN). To test the stability of **2a** to a weak acid silica gel (~5 mg) was added to a solution of **2a** (~5 mg in 0.6 mL $CDCl_3$) and the mixture was monitored by ^1H NMR. After 24 h, 2% of quinone methide **2a** could be seen in the ^1H NMR spectrum. As a result of this experiment, the stability of **2a** to protic acids was investigated. Thiol adduct **2a** was treated with a variety of buffer solutions (Scheme 1) under controlled conditions (~0.05 M, 1:1 buffer solution/ CH_3CN, pH 5-pH 9, 24 h, 37-39 °C, N_2 atmosphere). Extraction of the reaction mixture with $CHCl_3$, followed by concentration afforded the following mixtures of **1a** and **2a**: pH 5, **1a** (>1%), **2a**, (<99%); pH 6-7, **1a** (1%), **2a**, (99%); pH 8, **1a** (4%), **2a** (96%): pH 9, **1a** (6%), **2a** (94%). In each case the mass recovery was >90%. In order to ensure that the ratios of **1a:2a** were unchanged by the workup procedure, a reaction was carried out in CD_3CN/D_2O at pD 7. The ^1H NMR spectrum of the reaction mixture showed 1% of quinone methide **1a** was formed. Workup as described above also afforded the same ratio. At higher pH, more of quinone methide **1a** is formed. The stability of **2a** at pH 5-7 is notable.

Scheme 1. Stability of 2a vs pH.

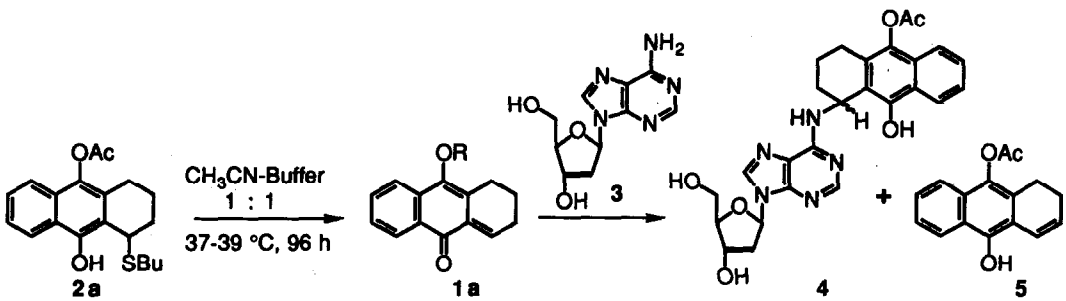


The above results lead us to examine the possible exchange of a thiol for a nucleoside under the same conditions. The results will provide some information as to the relative thermodynamic stability of thiol-quinone methide and nucleoside-quinone methide adducts at different pH's. Given the small amounts of quinone methide **1a** seen at pH 7, it might seem unreasonable to expect a nucleoside to be exchange for a thiol. The results were quite surprising.

Accordingly, thiol adduct **2a** was treated with 2'-deoxyadenosine, **3**, (1 equiv) in a 1:1 mixture of buffer solution[9]/CH_3CN at 37-39 °C for 96 hours (Scheme 2). The yields reported refer to isolated yields of material obtained by flash chromatography. At pH 5 only recovered thiol adduct **2a** was obtained, no nucleoside adduct **4** was formed. At pH 6, 1% of 2'-deoxyadenosine adduct **4**[7b] was isolated by chromatography and 95% of starting material **2a** was recovered. The reaction at pH 7 afforded a 7% yield of **4**,[7b] 72% of starting material **2a** was recovered, and 12% of styrene **5** was formed.[10,11] The styrene was presumably formed by enolization of the quinone methide.[11] Pure diastereomers of adduct **4** do not equilibrate when resubmitted to the reaction conditions

Reaction of **2a** with **3** at pH 8 and pH 9 did not afford nucleoside adduct **4**, and resulted in low recovery of starting material, **2a**. The reaction at pH 8 afforded styrene **5** and water-quinone methide

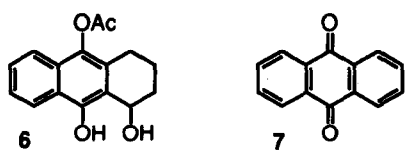
Scheme 2. Reaction of 2a with 3 vs pH.



Buffer pH	2a	4	5
5	99%	-	-
6	95%	1%	-
7	72%	7%	12%

Isolated yields after flash chromatography.

adduct **6** in a 1:1 ratio (^{1}H NMR analysis) as the sole products of the reaction. The reaction at pH 9 afforded anthraquinone **7** in 84% yield, again no starting **2a** was recovered. It is likely that compound **7** is derived from acetate hydrolysis and aromatization of styrene **5**. It is interesting to note that in absence of nucleoside, reaction of **2a** at pH 8 and pH 9 resulted in the recovery of starting material **2a** (94-96%).



These results show the stability and reactivity of thio-quinone methide adduct **2a** is highly pH dependent. In addition, our results show that a thiol might serve as a carrier for a quinone methide. For example, formation of a quinone methide followed by alkylation of a cysteinyl thiol group might afford a cysteine bound quinone methide that could later lead to regeneration of the quinone methide. Indeed, this might lead to the inability of scavengers for alkylating agents, such as glutathione to inactivate the quinone methide. The preference for the formation of a nucleoside adduct in the presence of an equimolar amount of thiol point to an inherent stability to the quinone methide-nucleoside adduct. This stability might be derived from favorable intramolecular hydrogen bonding or π-stacking interactions.

ACKNOWLEDGEMENTS

This work was supported by funds provided by the National Institutes of Health (GM 39354). We thank Mr. Ron New and Dr. Richard Kondrat for determination of mass spectra.

REFERENCES

1. (a) Moore, H. W. *Science* **1977**, *197*, 527. (b) Moore, H. W.; Czerniak, R. *Med. Res. Rev.* **1981**, *1*, 249. (c) Lin, A. J.; Sartorelli, A. C. *J. Med. Chem* .**1976**, *19*, 1336 and references cited therein. (d) For current work : Lin, T.-S.; Antonini, I.; Cosby L. A.; Sartorelli, A. C. *J. Med. Chem* **1984**, *27*, 813 and references cited therein.
2. (a) Boldt, M.; Guadiano, G.; Haddadin, M. J.; Koch, T. H. *J. Am. Chem. Soc.* **1989**, *111*, 2283, **1988**, *110*, 3330, and references cited therein. (b) Egholm, M.; Koch, T. H. *Ibid*, **1989**, *111*, 8291.
3. (a) Kleyer, D. L.; Gaudiano, G.; Koch, T. H. *J. Am. Chem. Soc.* **1984**, *106*, 1105. (b) Kleyer, D. L.; Koch, T. H. *Ibid* **1984**, *106*, 2380. (c) Olson, J. B.; Koch, T. H. *Ibid* **1986**, *108*, 756, and references cited therein.
4. (a) Ramakrishnan, K.; Fisher, J. F. *J. Med. Chem.* **1986**, *29*, 1215. (b) Fisher, J. F.; Abdella, B. R. J.; McLane, K. E. *Biochemistry* **1985**, *24*, 3562. (c) Fisher, J. F.; Aristoff, P. A. *Prog. Drug Res.* **1988**,*32*, 411.
5. (a) Anne, A.; Moiroux, J. *Nouv. J. Chim.* **1985**, *9*, 83. (b) Land, E. J.; Mukherjee, T.; Swallow, A. J.; Bruce, J. M. *Arch. Biochem. Biophys.* **1983**, *225*, 116. (c) Land, E. J.; Mukherjee, T.; Swallow, A. J.; Bruce, J. M. *Br. J. Cancer* **1985**, *51*, 515.
6. (a) Abdella, B. R. J.; Fisher, J. F. *Environ. Health Perspect.* **1985**, *64*, 3. (b) Powis, G. *Pharmacol. Ther.* **1987**, *35*, 57.
7. (a) Angle, S. R.; Yang, W *J. Am. Chem. Soc.* **1990**, *112*, 4524. (b) Angle, S. R.; Yang, W. *J. Org. Chem.* **1992**, *57*, 1092.
8. Compound **2a** is a yellow solid: mp 102-103 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.30 (dd, *J* = 7.3, 1.7 Hz, 1 H, ArH), 7.63 (dd, *J* = 8.0, 1.1 Hz, 1 H, ArH), 7.41-7.51 (m, 3 H, 2 ArH, 1 OH), 4.31 (bs, 1 H, benzylic methine hydrogen), 2.86 (bs, 1 H), 2.76-2.67 (m, 1 H), 2.63-2.54 (m, 1 H) 2.46 (s, 3 H), 2.20 (bm, 1 H), 2.06 (bm, 2 H), 1.87 (bm, 1 H), 1.79-1.60 (m, 2 H), 1.53-1.41 (m, 2 H), 0.96 (t, *J* = 7.3 Hz, 3 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 169.15, 149.16, 137.40, 126.95, 126.89, 126.61, 124.83, 124.44, 122.60, 120.19, 116.50, 40.91, 31.78, 31.01, 29.22, 24.20, 22.15, 20.47, 18.03, 13.57; IR (CCl_4) 3284, 2936, 1757, 1596, 1573, 1445, 1371, 1210, 1183, 1060 cm^{-1}; MS (CI, CH_4) *m/z* 344(M^+, 3), 255 (27), 212 (79), 197 (10), 195 (10), 90 (32), 57 (100); HRMS calcd for $C_{20}H_{24}O_3S$ 344.1446, found 344.1466.
9. Buffer solutions were prepared as follows: (a) pH 5, 14.0 g KH-Phthalate and 2.7 g $NaHCO_3$ per liter in water. (b) pH 6, 23.2 g KH_2PO_4 and 4.3 g Na_2HPO_4 per liter in water. (c) pH 7, 9.1 g KH_2PO_4 and 18.9 g Na_2HPO_4 per liter in water. (d) pH 8, 11.8 g boric acid and 9.1 g borax per liter in water. (e) 6.2 g boric acid and 38.1 g borax per liter in water. The pH is unchanged by CH_3CN.
10. It is interesting to note that at pH 5, 6, and 7, no reaction was observed at 25 °C.
11. The structure of styrene **5** was supported by the ^{1}H NMR spectrum (300 MHz, $CDCl_3$) δ 7.94 (dd, *J* = 8.5, 1.3 Hz, 1 H, ArH), 7.63 (dd, *J* = 7.6, 1.1 Hz, 1 H, ArH), 7.45-7.33 (m, 2 H, ArH), 6.75 (dt, *J* = 9.9, 1.9 Hz, 1 H, ArC<u>H</u>=C), 6.09 (dt, *J* =10.9, 4.7 Hz, 1 H, ArCH=C<u>H</u>), 5.54 (s, 1 H, Ar<u>OH</u>), 2.77-2.69 (m, 2 H), 2.48 (s, 3 H), 2.31 (m, 2 H).

(Received in USA 15 June 1992; accepted 20 July 1992)