2,3-Dimethyl-5,6-bis(methylene)-1,4-benzoquinone. The Active Intermediate of Bioreductive Alkylating Agents

AI JENG LIN* AND ALAN C. SARTORELLI

Department of Pharmacology, Yale University School of Medicine, New Haven, Connecticut 06510

Received August 24, 1972

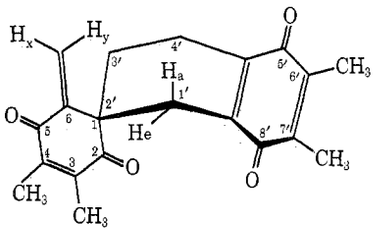
2,3-Dimethyl-5,6-bis(acetoxymethyl)-1,4-benzoquinone (**1**) has been shown by this laboratory to be a potent inhibitor of (a) the biosynthesis of DNA and RNA and (b) the growth of adenocarcinoma 755 ascites cells. It was hypothesized[1] that the action mechanism of this series of benzoquinone derivatives involved bioreduction *in vivo* in a manner analogous to mitomycin C by an NADPH-dependent quinone reductase enzyme[2,3] to corresponding dihydroquinones which generated reactive intermediates, *o*-quinone methides. Further postulation visualized that such active species might function as inhibitors of neoplastic growth by the alkylation of DNA, RNA, or other biological systems. In the present report, chemical evidence is presented to substantiate the formation of intermediate **3** from compound **1** upon sodium borohydride reduction.

Compound **1** was subjected to reduction with 1 molar equiv of $NaBH_4$ in methanol at ice-cold temperature. Two major yellow products were obtained after column chromatography on silica gel. The first compound was identified as duroquinone[4] (**4**) by nmr, ir, and mixture melting point with authentic sample. The second compound (**5**) had a parent ion peak at *m/e* 324 in the mass spectra and good elemental analysis based on the calculation of $(C_{10}H_{10}O_2)_2$, indicating that compound **5** was a dimer of **3** ($C_{10}H_{10}O_2$). Dimerization or trimerization were the most common reactions of *o*-quinone methides in the absence of other trapping reagents.[5] The structures of the dimer and trimer were in general a product of a Diels–Alder reaction of the *o*-quinone methides. There are relatively large numbers of possible dimer adducts which could be derived from the active intermediate **3**; however, only compound **5** (3′,4′-dihydro-3,4,6′,7′-tetramethyl-6-methylenespiro-[3-cyclohexene-1,2′(1′*H*)-naphthalene]-2,5,5′,8′-tetradone) best fits both nmr (Table I) and ir data. Characteristic benzoquinone peaks (KBr) around 1635 cm^{-1} and α,β-unsaturated keto groups at 1665 and 1675 cm^{-1} were observed in the ir spectrum.

In ^1H nmr spectra, the two most downfield signals (5.21 and 6.09 ppm) are assigned to the two olefinic protons. No coupling between these two protons was observed in either 60- or 100-MHz spectra, since the geminal coupling constant of olefinic protons is generally very small. The signal at lower field (6.09 ppm) is assigned to the proton (H_x) cis to the keto group of the ring, and arises from the anisotropic deshielding effect of the carbonyl.[6,7] The most upfield signals (1.87 ppm) appear as a triplet (J = 7.0 Hz) and integrate for two protons. Based on the splitting pattern and the coupling constant, this triplet is apparently part of the spectrum of an A_2B_2 system and therefore is assigned as either the $C_{3'}$ or $C_{4'}$ protons. The other part of the system is complicated by coupling with other protons to give nine peaks centered around 2.37 ppm. The splitting pattern of H_a and H_e indicates a large geminal coupling (J = 19 Hz) between H_a and H_e and a long-range coupling (J = 2.5 Hz) with either $C_{3'}$ or $C_{4'}$ protons. The coupling between the $C_{1'}$ and $C_{3'}$ protons can occur only when the system assumes a ω configuration, with the coupling protons approximately coplanar and linked by a zig-zag path.[8,9] The magnitude of this long-range coupling over four bonds ($^4J_{HH}$) is generally small and is often seen only in the broadening of a proton signal. Since only the H_e proton can assume the required ω configuration with the $C_{3'}$ protons, and long-range coupling is observed with both $C_{1'}$ protons, this coupling is probably derived from the homoallylic[8] long-range coupling between the $C_{1'}$ and $C_{4'}$ protons. Therefore, the triplet signal at 1.87 ppm is unambiguously assigned as $C_{3'}$ and the multiplets at 2.37 ppm assigned as $C_{4'}$. A closely related example of five-bond coupling between the protons of C_2 and C_5 of 1,2,5,6-tetrahydropyridine has been reported.[10] Using high-resolution nmr and decoupling techniques the coupling constant $J_{2,5}$ of 1,2,5,6-tetrahydropyridine was found to be 3 Hz, which is close to the value $J_{1'4'}$ = 2.5 Hz observed for $C_{1'}$ and $C_{4'}$ protons of dimer **5**.

The molecular model of dimer **5** indicated a serious steric interaction between H_y and $C_{1'}$ and $C_{3'}$ protons. Therefore, the most stable conformation of the molecule presumably is the one in which the ethylene group occupies an equatorial position at $C_{2'}$. In this conformation, the H_e proton, which is trans to the ethylene

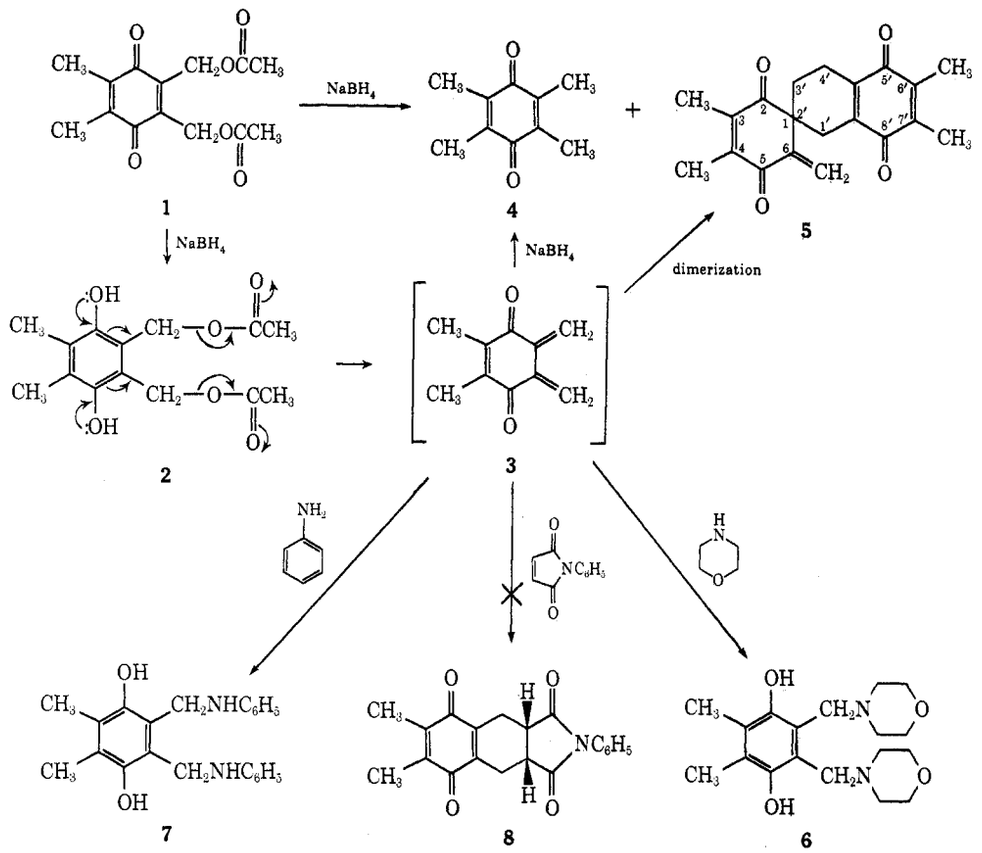
TABLE I[a]



Protons	Chemical shift, ppm	Coupling constants, Hz
$H_{3'}$	1.89 (t, 2)	$J_{3'4'}$ = 7
3,4,6′,7′-Tetramethyl	2.01 (s, 6), 2.06 (s, 6)	
$H_{4'}$	2.37 (m, 2)	$J_{4'3'}$ = 7, $J_{4'1'}$ = 2.5
H_e	2.65 (m, 1)	J_{ea} = 19, $J_{e4'}$ = 2.5
H_a	3.32 (m, 1)	J_{ae} = 19, $J_{a4'}$ = 2.5
H_y	5.21 (s, 1)	
H_x	6.09 (s, 1)	

[a] Chemical shifts and coupling constants from spectra of about 10% solutions in $CDCl_3$.

(1) A. J. Lin, L. A. Cosby, C. W. Shansky, and A. C. Sartorelli, *J. Med. Chem.*, **15**, 1247 (1972).
(2) H. S. Schwartz, J. E. Sodergren, and F. S. Philips, *Science*, **142**, 1181 (1963).
(3) V. N. Iyer and W. Szybalski, *ibid.*, **145**, 55 (1964).
(4) L. I. Smith and F. J. Dobrovolny, *J. Amer. Chem. Soc.*, **48**, 1420 (1926).
(5) A. B. Turner, *Quart. Rev., Chem. Soc.*, **18**, 347 (1964).
(6) J. W. ApSimon, W. G. Craig, P. V. Demarko, D. W. Mathieson, A. K. G. Nasser, L. Saunders, and W. B. Whalley, *Chem. Commun.*, 754 (1966).
(7) G. J. Karabatsos, G. C. Sonnichsen, N. Hsi, and D. J. Fienoglio, *J. Amer. Chem. Soc.*, **89**, 5067 (1967).
(8) S. Sternhell, *Rev. Pure Appl. Chem.*, **14**, 15 (1964).
(9) S. Sternhell, *Quart. Rev., Chem. Soc.*, **23**, 236 (1969).
(10) J. N. Shoolery, *Discuss. Faraday Soc., No. 34*, 104 (1962).



group, assumes a ω configuration with the equatorial $C_{3'}$ proton required for a four-bond long-range coupling. Consequently the H_e proton appeared as a relatively poorly resolved sextet, which contrasts with the sharply resolved sextet of the H_a proton. The downfield shift of the H_a proton can be explained by a steric compression effect caused by the overcrowding at the axial position.[11]

The mechanism of the formation of the observed products **4** and **5** can best be explained by the initial reduction of compound **1** with $NaBH_4$ to the corresponding dihydrobenzoquinone **2**, which then decomposed to generate **3**. Further reduction or dimerization of **3** produced the observed products **4** and **5**. The formation of a similar dimer, spirodi-*o*-xylylene, from *o*-xylylene has been documented.[12] To provide additional evidence for the existence of **3**, the reduction of **1** with $NaBH_4$ was carried out in the presence of morpholine or aniline. The expected adducts **6** or **7**, respectively, precipitated from the reaction mixture in 5–10 min after the addition of $NaBH_4$. A similar reaction mechanism which involved quinone methide as the intermediate was suggested to account for the formation of 2,5-bis(morpholinomethyl)-1,4-dihydrobenzoquinone by treatment of 2,5-dihydroxy-α,α'-*p*-xylylene bisisothiuronium dihydrochloride with morpholine.[13] However, an alternative mechanism which involves amination of quinone **1** followed by $NaBH_4$ reduction to give adducts **6** or **7** must be considered. Further experiments, therefore, were carried out in which benzoquinone **1** was treated with either morpholine or aniline in the absence of $NaBH_4$ under ice-cold temperature. No appreciable reaction occurred over 2 hr with these reaction conditions, as monitored by thin layer chromatography. This finding tends to eliminate the possibility of an amination–reduction mechanism, and further substantiated a mechanism involving the generation of *o*-quinone methide (**3**) as the active intermediate.

Attempts to trap intermediate **3** in a similar manner with *N*-phenylmaleimide failed to give the desired adduct **8**. Instead, compounds **4** and **5** were isolated. In view of the numerous examples of the susceptibility of imides to $NaBH_4$ reduction,[14] the failure to trap intermediate **3** with *N*-phenylmaleimide may be due to the rapid reductive destruction of the trapping agent. However, identical results were obtained using ω-nitrostyrene, which would be expected to be stable under the reduction conditions employed. These findings do not necessarily disprove the existence of intermediate **3**, but rather that it is a better dienophile than trapping agents in the competitive Diels–Alder reaction.[15]

In summary, evidence was obtained to indicate that the reactive intermediate 2,3-dimethyl-5,6-bis(methylene)-1,4-benzoquinone (**3**) was formed from 2,3-dimethyl-5,6-bis(acetoxymethyl)-1,4-benzoquinone (**1**) upon $NaBH_4$ reduction. To the best of our knowledge, this is the first report of evidence supporting the existence of bis(*o*-quinone methide). The capability of intermediate **3** to alkylate aniline or morpholine suggested a similar potential to alkylate biological materials *in vivo*, if **3** could be generated enzymatically *in vivo*.

(11) T. Sato and K. Uno, *Chem. Commun.*, 579 (1972).
(12) L. A. Errede, *J. Amer. Chem. Soc.*, **83**, 949 (1961).
(13) D. L. Fields, J. B. Miller, and D. D. Reynolds, *J. Org. Chem.*, **30**, 3962 (1965).
(14) Y. Kondo and B. Witkop, *ibid.*, **33**, 206 (1968).
(15) H. L. Holmes, "Organic Reactions," Vol. IV, Wiley, New York, N. Y., 1948, Chapter 2, p 60.

Experimental Section

$NaBH_4$ Reduction of 2,3-Dimethyl-5,6-bis(acetoxymethyl)-1,4-benzoquinone.—Quinone 1 (0.5 g, 1.8 mmol) was suspended in 20 ml of methanol and chilled in an ice bath. $NaBH_4$ (0.065 g, 1.8 mmol) was added in small portions to the suspension with stirring. The clear yellow solution was stirred at ice-cold temperature for another 30 min after the addition of $NaBH_4$. The methanol was evaporated to dryness under reduced pressure and room temperature to give a yellow powder. Water (20 ml) was added and the mixture was extracted three times with ether (30 ml). Ether extracts were combined, dried, and evaporated to dryness. The yellow powder was chromatographed on a column of silica gel (50 g) using EtOAc and petroleum ether (bp 38–47°) (1:4, v/v) as eluent; two major yellow fractions were obtained. The first fraction yielded 50 mg of long needles (from H_2O), mp 110°, and was identified as duroquinone (4) (lit.[4] mp 111°) by ir, nmr, and mixture melting point. The second fraction yielded 75 mg of dimer 5: mp 155.5–157.5°; ir (KBr) 1635, 1665, and 1675 cm^{-1} (-C=O); mass spectrum *m/e* 324 (M^+); uv λ_{max}^{EtOH} 259 nm (ϵ 27,000), 265 (24,000).

Anal. Calcd for $C_{20}H_{20}O_4$: C, 74.07; H, 6.17. Found: C, 73.88; H, 6.31.

$NaBH_4$ Reduction of 2,3-Dimethyl-5,6-bis(acetoxymethyl)-1,4-benzoquinone in the Presence of Morpholine.—Quinone 1 (0.25 g, 0.9 mmol) and morpholine (0.5 ml) were suspended in 10 ml of ice-cold methanol. To the suspension, $NaBH_4$ (0.035 g, 0.9 mmol) was added in small portions with stirring. After the addition, the solution was mixed for an additional 30 min. The white precipitate was collected and washed with ice-cold methanol to give 60 mg of white crystals. The filtrate was evaporated to dryness and the crude product was washed with H_2O followed by cold methanol to give another 50 mg of white crystals. Combination and recrystallization of the product from methanol yielded 100 mg (37%) of white crystals (6): mp 206° dec; ir (KBr) 3450–2700 (broad and weak, -OH···N-) and 1110 cm^{-1} (ether); nmr ($CDCl_3$) 2.71 (s, 6), 2.54 (m, 8), 3.68 (s, 4) and 3.75 ppm (m, 8).

Anal. Calcd for $C_{18}H_{28}N_2O_4$: C, 64.29; H, 8.33; N, 8.33. Found: C, 64.01; H, 8.26; N, 8.28.

$NaBH_4$ Reduction of 2,3-Dimethyl-5,6-bis(acetoxymethyl)-1,4-benzoquinone in the Presence of Aniline.—To quinone 1 (0.15 g, 0.53 mmol) in 20 ml of methanol was added an excess of aniline (0.5 ml). The solution was cooled in an ice bath and $NaBH_4$ (0.02 g, 0.53 mmol) was added in small portions. The clear solution was stirred for 10 min and the formed white precipitate was collected and washed with a small amount of methanol. Recrystallization from ethanol gave white needles of 7 (0.14 g, 76%): mp 171–173° dec; ir (KBr) 3290 (s, NH), 3200–2700 (-OH···N-), 1602, 1500, 747, and 690 cm^{-1} (monosubstituted phenyl); nmr (DMSO-d_6) 2.10 (s, 6), 4.25 (broad singlet, 4), 5.43 (broad singlet, 2), 6.88 (m, 10), 8.13 ppm (broad singlet, 3).

Anal. Calcd for $C_{22}H_{24}N_2O_2$: C, 75.85; H, 6.90; N, 8.05. Found: C, 75.73; H, 7.02; N, 7.79.

Registry No.—1, 37439-56-8; 3, 37439-57-9; 5, 37439-58-0; 6, 37439-59-1; 7, 37439-60-4; $NaBH_4$, 16940-66-2; morpholine, 110-91-8; aniline, 62-53-3.

Acknowledgment.—This study was supported by Grant CA-02817 from the National Cancer Institute, USPHS.

Hydrolysis Products of 4-Acetamido-4-hydroxy-2-butenoic Acid γ-Lactone

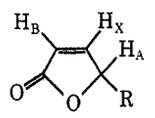
MICHAEL D. GROVE* AND DAVID WEISLEDER

Northern Regional Research Laboratory,[1] Peoria, Illinois 61604

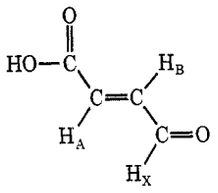
Received August 17, 1972

4-Acetamido-4-hydroxy-2-butenoic acid γ-lactone (**1a**) is a mycotoxin produced on laboratory media by a strain of *Fusarium tricinctum* originally isolated from tall fescue hay (*Festuca arundinaceae* Schreb.).[2] Gangrene in tails of cattle receiving **1a** provided circumstantial evidence implicating it in the problem of tall fescue toxicity.[3,4]

Our current interest in the biological activity of **1a** prompted us to examine more closely two earlier reports concerning the hydrolysis products of this mycotoxin. White[5] found that acid hydrolysis of **1a** gave a 23% yield of malealdehydic acid (*cis*-β-formylacrylic acid, **1b**) as the major four-carbon fragment isolated; alkaline hydrolysis also gave **1b**, no yield being reported. Burkhardt, *et al.*,[6] found that alkaline hydrolysis of **1a** yielded a mixture of **1b** and fumaraldehydic acid (*trans*-β-formylacrylic acid, **2**). However, their results were



1a, R = $NHCOCH_3$
b, R = OH
c, R = OCH_2CH_3



2

not unambiguous since the melting point given for cis acid **1b** (127°) is the same as reported by Schroeter, *et al.*,[7] for trans acid **2**. Our investigation shows that, whereas acid hydrolysis of **1a** does indeed give **1b** as the major product, **2** predominates under alkaline conditions. Hydrolysis products were identified by direct comparison with unequivocally characterized samples of **1b** and **2**.

Compound **1b** had been prepared earlier[7] by acid hydrolysis of the corresponding ethyl pseudo ester **1c**, which was obtained by photosensitized oxygenation of furfural in ethanol.[8] We prepared **1b** more directly, albeit in lower yield (25%), by carrying out the photooxygenation of furfural in aqueous ethanol (1:1). The ethanol required to maintain the eosin sensitizer in solution leads to formation of some **1c**. The ir spectrum of **1b** exhibits a pair of carbonyl bands at 1790 and 1760 cm^{-1} characteristic of an α,β-unsaturated lactone of this type.[2] That **1b** exists as the cyclic pseudoacid and not as an open-chain aldehydic acid as its name implies was confirmed by the nmr spectrum (Table I), which shows no aldehyde proton. The three ring protons of **1b** exhibit an ABX pattern with the cis-vinyl proton coupling constant of 5.7 Hz in close agreement with that for **1a**[6] and *cis*-β-acetylacrylic acid.[9]

Compound **2** had been prepared previously by acid treatment of either **1b** or **1c** in 25 and 46% yields, re-

(1) Agricultural Research Service, U. S. Department of Agriculture.

(2) S. G. Yates, H. L. Tookey, J. J. Ellis, and H. J. Burkhardt, *Phytochemistry*, **7**, 139 (1968).

(3) M. D. Grove, S. G. Yates, W. H. Tallent, J. J. Ellis, I. A. Wolff, N. R. Kosuri, and R. E. Nichols, *J. Agr. Food Chem.*, **18**, 734 (1970); H. L. Tookey, S. G. Yates, J. J. Ellis, M. D. Grove, and R. E. Nichols, *J. Amer. Vet. Med. Ass.*, **160**, 1522 (1972).

(4) For a recent review see S. G. Yates in "Microbial Toxins," Vol. 7, S. Kadis, A. Ciegler, and S. J. Ajl, Ed., Academic Press, New York, N. Y., 1971, pp 191–206.

(5) E. P. White, *J. Chem. Soc. C*, 346 (1967).

(6) H. J. Burkhardt, R. E. Lundin, and W. H. McFadden, *Tetrahedron*, **24**, 1225 (1968).

(7) S. H. Schroeter, R. Appel, R. Brammer, and G. O. Schenck, *Justus Liebigs Ann. Chem.*, **697**, 42 (1966).

(8) G. O. Schenck, *ibid.*, **584**, 156 (1953).

(9) S. S. Seltzer and K. D. Stevens, *J. Org. Chem.*, **33**, 2708 (1968).