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Model Reactions of the Quinone Metabolites of Carcinogenic Hydrocarbons with t-Butylthiol

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Reactions of benzo[*a*]pyrene1,6-dione with t-butylthiolate affords two products of conjugate addition and subsequent spontaneous reoxidation, namely, 2-t-butylthio- and 2,4-di-t-butylthiobenzo[*a*]pyrene 1,6-dione. Analogous reaction of benzo[*a*]pyrene 3,6-dione furnished only 12-t-butylthiobenzo[*a*]pyrene 3,6-dione. Structural assignments are based on analysis of the high-resolution 270-MHz Fourier-transform proton nmr spectra. In both products the attachment of the entering nucleophile is on an aromatic ring remote from either of the carbonyl groups, the first examples of such detected. The biological significance of these results with relation to the potential reactions of these quinones, known to be major metabolites of the carcinogen benzo[*a*]pyrene, with glutathione, cysteine, and proteins *in vivo* is discussed.

INTRODUCTION

Quinones are among the principal metabolites of carcinogenic polycyclic aromatic hydrocarbons (*1*, *2*,). Although it is commonly assumed that these derivatives are not involved in the induction of cancer, the 1,6-, 3,6-, and 6,12-diones of benzo[*a*]pyrene (BP) are highly cytotoxic to cells in culture (*3*), and incubation of these quinones with T7 DNA induces strand breakage of the nucleic acid which is oxygen and NADH dependent (*3–5*).

Simple quinones are known to react readily with mercaptans, including both cysteine and glutathione, to afford products of 1,4-addition and subsequent reoxidation to substituted quinones (*6*). Similar reaction has not been demonstrated for the polycyclic quinone metabolites. However, hydrocarbon metabolites are known to interact *in vivo* with proteins to afford products covalently attached to the thiol group of cysteine (*7*, *8*).

We report now on the reactions of the 1,6-diones of BP with t-butylthiolate. Our previous studies have demonstrated the suitability of this reagent as a model for cysteine (*7*), glutathione, and guanosine (*9*) in reactions with arene oxides (*10*) and benzo[*a*]-pyrene diolepoxide (*11*). In this study, we sought to determine the regioselectivity of reaction and to obtain nmr and other data on the products to aid in the eventual structural assignment of the more complex products anticipated to be formed from glutathione, cysteine, and proteins.

RESULTS

Reactions of the quinones with t-butylthiolate were conducted at ambient temperature in alkaline aqueous dioxane under nitrogen atmosphere. Under these

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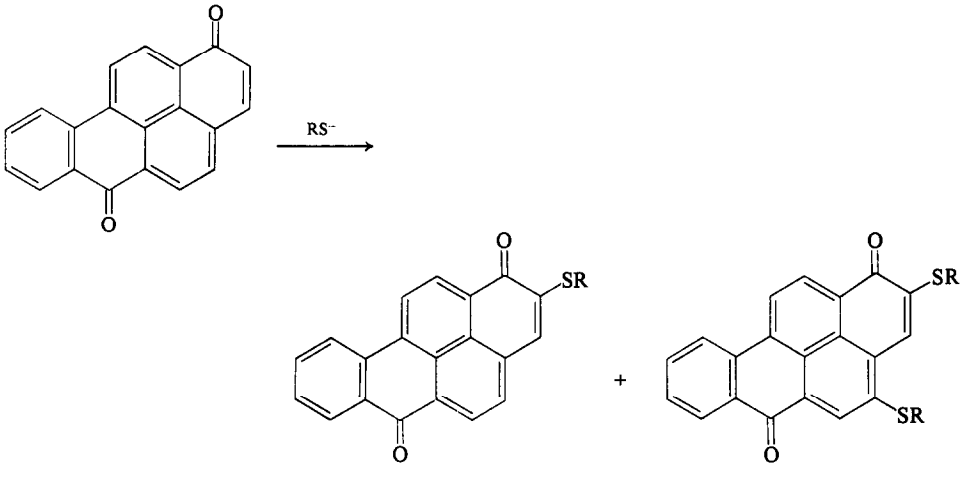
conditions, reaction of BP 1,6-dione afforded two principal products isolated by hplc on silica. Their infrared spectra exhibited carbonyl and other peaks consistent with quinone-type structures. Microanalysis indicated the major product to be a mono-t-butylthio-BP-dione and the minor product to be a di-t-butylthio BP-1,6-dione.

The structural assignment of these compounds as 2-t-butylthiobenzo[*a*]pyrene 1,6-dione (**1**) and 2,4-di-t-butylthiobenzo[*a*]pyrene 1,6-dione (**2**) was made through analysis of their 270-MHz high-resolution FT-nmr spectra. These assignments were facilitated by the prior demonstration (*10*) that the t-butylthio substituent induces downfield shifts of the nearby peri aromatic protons of average $\Delta\delta = 1.0$ ppm, while the adjacent ortho aromatic protons were displaced by average $\Delta\delta = 0.5$ ppm.



1 **2**

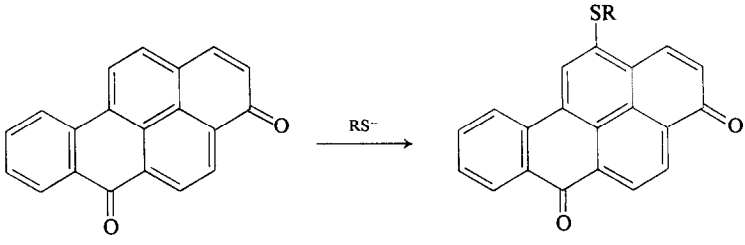
R = $C(CH_3)_3$

Thus the nmr spectrum of **1** closely resembled that of the parent quinone (*12*), the only significant differences being the presence of the t-butyl singlet peak at δ 1.53 and replacement of the characteristic *AB* pattern of the vinylic protons by a sharp singlet for a single vinylic proton at δ 7.87. Therefore, the t-butylthio group must be located at C-2 or C-3. Since the H_4 proton which is peri to C-3 does not exhibit the major downfield shift anticipated if the substituent were in the adjacent 3-position, it may be concluded that the thioether group is a C-2. This assignment is supported by the observed chemical shift of H_3 (δ 7.87) which is displaced ~0.3 ppm downfield from the corresponding proton of BP 1,6-dione. This shift is consistent with its location at C-3 ortho to the sulfur group (*11*) and inconsistent with the reverse assignment (*13*). Thus, the nmr spectral data are consistent with only 2-t-butylthiobenzo[*a*]pyrene 1,6-dione (**1**) for the structure of the monosubstituted BP–1,6-dione product.

The structural assignment of **2** was made on similar grounds. The nmr spectrum of **2** exhibited chemical shifts and couplings similar to those of BP 1,6-dione for the protons H_{7-12}. The remaining protons appeared as singlets at δ 1.42 (9*H*) and 1.57 (9*H*) for the t-butyl protons and two additional singlets at δ 8.75 (1*H*) and 8.87 (1*H*). Since these latter signals exhibit no couplings, the related protons must reside on different rings in the 2,3,4, or 5 positions. Therefore, one of these must be a vinylic proton, despite the

rather dramatic downfield shift. The latter is explicable only if the substituents are located at C-2 and C-4. In this case, H_3 may be expected to appear ~1.0 ppm downfield from H_3 of **1** due to the effect of the sulfur group in the flanking peri position (i.e., δ 7.87 + 1.0 = 8.87), which agrees closely with observation. Therefore, the structure of the disubstituted product is 2,4-di-t-butylthiobenzo[*a*]pyrene 1,6-dione (**2**).

Analogous reaction of BP 3,6-dione furnished a singlet product shown by infrared and microanalysis to be a t-butylthio-BP 3,6-dione. The position of substitution was not at C-1 or C-2, since these protons gave a normal *AB* pattern with characteristic coupling ($J_{1,2}$ = 10.2 Hz) in the nmr spectrum. However, H_1 appeared at unexpectedly low field (δ 8.79), indicating the presence of the t-butylthio group in the adjacent 12 position. In accord with this assignment, H_{11} appeared as a singlet downfield from the equivalent proton of the parent quinone ($\Delta\delta = 0.16$). The 12-t-butylthiobenzo[*a*]pyrene 3,6-dione (**3**) structure is, therefore, assigned to this compound.



$R = C(CH_3)_3$

DISCUSSION

These results demonstrate that the polycyclic BP quinones, like their simpler analogs (*6*), undergo conjugate addition of mercaptans and reoxidation to furnish substituted quinone products. The position of substitution, however, is quite dependent upon quinone structure. With BP 1,6-dione, 1,8-addition occurs initially to afford **1**, followed by 1,6-addition to furnish **2**. With BP 3,6-dione only 1,6-addition to afford **3** is detected. In two cases, therefore, the point of attachment of the entering nucleophile is on an aromatic ring remote from either of the carbonyl groups. These are the first examples (*6*), to our knowledge, where such is the case.

The biological significance of these results is not yet certain, but it appears rather likely that the quinone metabolites of BP and other polycyclic hydrocarbons may react with cellular nucleophiles, such as glutathione and the cysteine components of proteins to form similar derivatives. Although basic conditions are required to assure satisfactory yields of the BP quinone addition products, it cannot be assumed that similar reactions are improbable under physiological conditions. For example, although arene oxides fail to react appreciably with simple thiol compounds at neutral pH (*14*, *15*), they are detoxified *in vivo* principally through reaction with the thiol group of glutathione catalyzed by the enzyme glutathione *S*-epoxide transferase (*2*).

Although reaction of the BP quinones with glutathione *in vivo* is likely to lead only to excretion, reaction with the cysteine component of a protein may have profound biological significance. The resulting protein-bound quinones could function as catalysts

linking NADH and O_2 in the production of H_2O_2 and related reactive oxygen species ($O_2^{\cdot-}$, HO·) (*3–5*), with the additional advantage of protection from normal detoxification by the partially enfolding protein structure. Thus, a small amount of protein-bound quinone could result in significant cellular damage which could conceivably lead to the induction of cancer.

EXPERIMENTAL SECTION

Materials and Methods

The BP 1,6- and 3,6-quinones were synthesized as previously described (*12*). t-Butylthiol was employed as supplied by Aldrich Co. Proton nmr spectra were obtained on Varian T-60 and Bruker 270-MHz spectrometers; chemical shifts are reported relative to Me_4Si in $CDCl_3$. The hplc separations were performed on a $\frac{1}{2} \times$ 24-in. column of silica (LiChrosorb 10μ) with hexane–CH_2Cl_2 (3:2) as solvent. A Variscan ultraviolet detector was employed. Microanalyses for C,H, and S correct to $\pm 0.3\%$ were obtained for all new compounds; see Table 1.

TABLE 1

MICROANALYTICAL DATA

	C		H		S	
Compound	Calcd	Found	Calcd	Found	Calcd	Found
1	77.81	77.52	4.90	4.94	8.65	8.54
2	73.33	73.22	5.71	5.92	13.98	13.79
3	77.81	77.54	4.90	4.93	8.65	8.50

Reaction of Benzo[a]pyrene 1,6-Dione with t-Butylthiol

To a solution of the quinone (50 mg, 0.18 mmol) in 50 ml of dioxane was added a solution of 141 mg of NaOH in 25 ml of water, followed by t-butylthiol (32 mg, 0.35 mmol). The resulting dark-colored suspension was stirred overnight at ambient temperature. Water was then added, and the product extracted with ether, washed three times with water, dried over $MgSO_4$, and evaporated to dryness *in vacuo*. The product (75 mg) was a red solid which gave on tlc on silica gel with ethyl acetate–benzene (1:1) a single spot with $R_f = 0.67$ free of the yellow BP 1,6-dione ($R_f = 0.52$). Passage through a column of Florisil (20 g) eluted with 9:1 benzene–ethyl acetate (300 ml) furnished the purified product (69 mg). The hplc on silica cleanly separated the latter into 2,4-di-t-butylthiobenzo[*a*]pyrene 1,6-dione (**2**) (13 mg, 16%) 2-t-butylthiobenzo[*a*]pyrene 1,6-dione (**1**) (35 mg, 53%) in order of elution. Recrystallization of the latter from benzene–hexane afforded the analytical sample of **1**, mp 232–233.5°C: ir broad peak 1650 cm^{-1} (C=O); nmr δ 1.53 (*s*, 9, CH_3), 7.56 (*d* of *t*, 1, H_9), 7.69 (*d*, 1, H_4), 7.71 (*d* of *t*, H_8), 7.87 (*s*, 1, H_3), 8.13 (*d* of *d*, 1, H_7), 8.28 (*d*, 1, H_5), 8.32 (*d* of *d*, 1, H_{10}), 8.43 (*d*, 1, H_{11}), and 8.46 ppm (*d*, 1, H_{12}); $J_{4,5} = 7.7$, $J_{7,9} \simeq 1$, $J_{8,9} \simeq 7.5$, $J_{8,10} \simeq 1$, $J_{9,10} = 8.3$, and $J_{11,12} = 8.1$.

Recrystallization of compound **2** from benzene–hexane afforded the analytical sample, mp 263.5–265.0°C: ir broad peak 1650 cm^{-1} (C=O); nmr (*16*) δ 1.42 (*s*, 9, CH_3), 1.57 (*s*, 9, CH_3), 7.60 (*d* of *t*, 1 H_9), 7.76 (*d of t*, 1, H_8), 8.26 (*d* of *d*, 1, H_7), 8.40 (*d* of *d*, 1, H_{10}), 8.46 (*d*, 1, H_{11}), 8.64 (*d*, 1, H_{12}), 8.75 (*s*, 1, H_5), and 8.87 ppm (*s*, 1, H_3); $J_{7,8} = 7.7$, $J_{7,9} = J_{8,10} = 1.5$, $J_{8,9} = 7.5$, $J_{9,10} = 7.5$, $J_{11,12} = 8.1$; MS, molecular ion $m/e = 458.1379$ ($C_{28}H_{26}S_2O_2 = 458.1374$).

Reaction of Benzo[a]pyrene 3,6-Dione with t-Butylthiol

Analogous reaction of the BP 3,6-dione (50 mg) with t-butylthiol (32 mg) afforded 98 mg of a rust-brown solid. Passage through a column of Florisil (20 g) eluted with 400 ml of ethyl acetate–chloroform (1:9) furnished 72 mg of a brownish-red solid. The hplc on silica gave 12-t-butylthiobenzo[*a*]pyrene 3,6-dione (**3**) (39 mg, 60%). Recrystallization from benzene provided pure **3**, mp 251.5–252.5°C; nmr δ 1.28 (*s*, 9, CH_3), 6.72 (*d*, 1, H_2), 7.60 (*d* of *t*, 1, H_9), 7.76 (*d* of *t*, 1, H_8), 8.23 (*d* of *d*, 1, H_{10}), 8.46 (*d* of *d*, 1, H_{10}), 8.51 (*s*, 1, H_{11}), 8.63 (*d*, 1, H_1), 8.71 (*d*, 1, H_4), and 8.79 ppm (*d*, 1, H_5); $J_{1,2} = 10.2$, $J_{4,5} = 7.7$, $J_{7,8} = 8.3$, $J_{7,9} \simeq 1$, $J_{8,9} \simeq 8$, $J_{8,10} \simeq 1$, and $J_{9,10} = 8.4$.

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