spectra. Spectra were simulated using Bloch equations modified for chemical exchange.[19]

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Effect of Bay Region Methyl Group on Reactions of *anti*-Benz[*a*]anthracene 3,4-Dihydrodiol 1,2-Epoxides with DNA†

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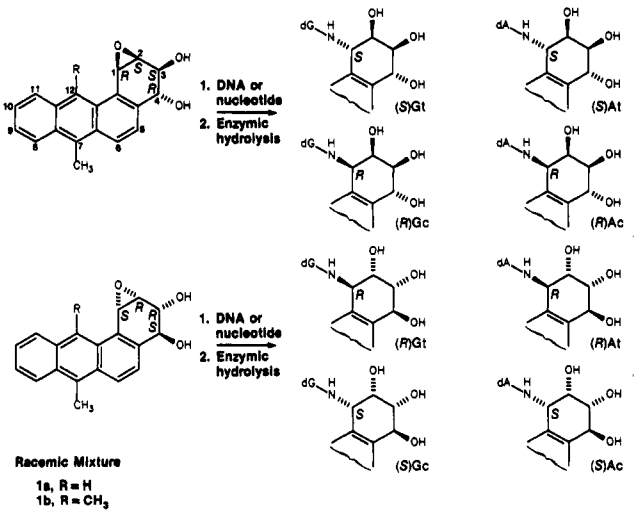
The NMR spectroscopic characterization of seven 7-methylbenz[*a*]anthracene–deoxyribonucleoside adducts and eight 7,12-dimethylbenz[*a*]anthracene–deoxyribonucleoside adducts, derived from the reaction of the corresponding *anti*-dihydrodiol epoxides and deoxyguanylic and deoxyadenylic acids, is described. The epoxide ring is opened by the purine amino groups to yield both cis and trans products from each enantiomer in the racemic dihydrodiol epoxides. Circular dichroism and NMR spectra allow the conformations of the products to be established. Interesting differences between the products from the two hydrocarbons are as follows: the dimethyl derivative is distributed fairly evenly over adenine and guanine residues in DNA, whereas guanine is the principal site for reaction of the monomethyl derivative; the conformation of the tetrahydro ring system is similar in trans products for both hydrocarbons with the hydrogens on C_3 and C_4 being pseudodiaxial; in cis adducts, these hydrogens are pseudodiaxial for 7,12-dimethylbenz[*a*]anthracene adducts but pseudodiequatorial for the 7-methylbenz[*a*]anthracene adducts; in reactions with nucleotides, trans adducts predominate for 7-methylbenz[*a*]anthracene derivatives but trans and cis adducts form to similar extents for 7,12-dimethylbenz[*a*]anthracene derivatives. This latter differs substantially from previous findings with other bay region substituted hydrocarbons where cis adducts have been obtained only in low yields.

Introduction

Polycyclic aromatic hydrocarbons express carcinogenic and mutagenic properties through covalent reactions with cellular DNA.[1] These reactions are mediated by metabolically generated bay region dihydrodiol epoxides[2,3] that aralkylate DNA following the chemistry first described for 7-(bromomethyl)benz[*a*]anthracene, i.e., the amino groups of the DNA bases are the principal sites of reaction.[4] However, the dihydrodiol epoxide chemistry is complex because of the different stereoisomers of the dihydrodiol epoxides and the fact that the epoxide ring can open to give cis or trans products.[5] Although the identity of the major adducts of the *anti*-dihydrodiol epoxides of benzo[*a*]pyrene was established soon after their discovery,[6] quantities of adducts from this[7] and other epoxides,[8] sufficient for detailed structural characterizations, have been prepared only recently through reactions of synthetic dihydrodiol epoxides with high concentrations of deoxy-

Scheme I. Structures of Adducts Formed in DNA and Nucleotide Reactions



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ribonucleotides. Thus, adducts from both the *syn* and *anti* bay region dihydrodiol epoxides of benzo[*c*]phenanthrene[8] and dibenz[*a*,*j*]anthracene[9] have been recently described, as have adducts from the *anti*-dihydrodiol epoxides of benzo[*a*]pyrene[7] and 5-methylchrysene[10] and from the *syn*-dihydrodiol epoxides of 7,12-dimethylbenz[*a*]-anthracene (DMBA).[11] Some interesting effects of hydrocarbon structure on adduct distribution and conformation have already been noted.[12]

The object of the present study was to evaluate the effect of a bay region methyl substituent adjacent to the angular ring upon dihydrodiol epoxide–DNA and –nucleotide reactions. It is known that such bay region methyl substituted hydrocarbons are generally much more potent carcinogens than the corresponding unsubstituted hydrocarbons,[13] and indeed, DMBA, the parent of one of the metabolites we have chosen to study, is generally regarded as the most potent tumor initiator amongst the hydrocarbon carcinogens,[1] and, though less potent,[14] 7-methylbenz[*a*]anthracene (7MeBA), the parent of the other metabolite studied herein, is also a powerful carcinogen.[15]

We have previously established that the (4*R*,3*S*)-dihydrodiol (2*S*,1*R*)-epoxide of DMBA is the only enantiomer of the *anti* diastereomer **1b** that mediates the binding of DMBA to DNA in cellular systems,[16] and we have utilized this finding to distinguish the diastereomers formed from the racemic dihydrodiol epoxide used in the present studies. We describe herein the characterization of *cis*- and *trans*-deoxyguanosine (G) and -deoxyadenosine (A) adducts arising from racemic dihydrodiol epoxides **1a** and **1b** (Scheme I) and show that the presence of the methyl group in the bay region in **1b** is associated with a substantially increased preference for reaction with adenine residues in DNA and with an increased tendency to form cis products and that cis products adopt different conformations in the bay region substituted and unsubstituted cases.

Experimental Section

Racemic *anti*-3,4-dihydrodiol 1,2-epoxides of 7-methylbenz[*a*]anthracene (**1a**) and of 7,12-dimethylbenz[*a*]anthracene (**1b**) were prepared as described.[17,18] Calf thymus DNA, deoxyribonucleotides, and enzymes were all obtained commercially.

CD spectra of nucleoside adducts in methanol were normalized to 1.0 absorbance unit at λ_{max}.

NMR spectra of acetylated tetraols and adducts were recorded in acetone-d_6. Spectra were acquired at 25 °C by signal averaging for up to 60 h using block averaging. Typical acquisition parameters were sweep width 5200 Hz, acquisition time 3 s, recycle time 4 s, pulse angle 30°. 2D-COSY spectra were obtained typically collecting 512 blocks of 1024 data points with acquisition time approximately 0.15 s, sine bell apodized and zero filled to 1K by 1K data set. A pulse angle of 90° was used for all COSY spectra. Nuclear Overhauser enhancement (NOE) experiments were performed by irradiation of individual resonances with a low-power, selective pulse for 6 s prior to acquisition. All NOE irradiations on individual samples were performed as a single experiment by creating a list of decoupler offsets and interleaving during acquisition. NOE experiments were performed nonspinning with an acquisition pulse angle of 90°. In certain instances, coupling constants were extracted from overlapping resonances by use of spectral simulation and resolution enhancement.

Structures for molecular modeling were generated using Primer version 3.0 and energy minimizations were accomplished using BatchMin version 3.1 of the software package MACRO MODEL.[19] The molecular force field utilized in this work was Allinger's MM2, and minimization was carried out *via* the Block Diagonal Newton Raphson method. The acetylated *cis*-**1a**–nucleoside adduct structures were subjected to free minimization to yield structures compatible with experimental NMR data. For the corresponding *cis*-**1b**–nucleoside adducts, the 12-methyl group was added to the **1a** series structure and the dihedral angles in the 1,2,3,4-ring were varied until the calculated coupling constants were consistent with the observed NMR coupling constants. A further free minimization was then carried out.

DNA Adduct Formation and Isolation. Calf thymus DNA (1 mg/mL) in Tris·HCl buffer (0.1 M, pH 7) was mixed with 0.1 volume of an acetone solution of racemic **1a** (1 mg/mL) or an acetone/ether/tetrahydrofuran (11/11/1) solution of freshly prepared racemic **1b** (0.87 mg/mL) and incubated at 37 °C overnight. After extraction with water-saturated 1-butanol (3 × 1 volume) followed by ether (2 × 1 volume) to remove dihydrodiol epoxide hydrolysis products, the pH of the aqueous phase was adjusted and the DNA was enzymically digested to nucleosides using snake venom phosphodiesterase (0.2 units per mg DNA) and alkaline phosphomonoesterase. The nucleoside–hydrocarbon adducts were recovered on Sep Pak Cartridges as described before.[7]

Deoxyadenosine and Deoxyguanosine Adducts. Solutions of deoxyadenylic (10 mg/mL) and deoxyguanylic acids (10 mg/mL) in Tris–HCl buffer (0.1 M, pH 7) were separately mixed with 0.1 volume of an acetone solution of racemic **1a** and were incubated overnight at 37 °C. Higher concentrations of nucleotides (40 mg/mL) were used in analogous reactions with freshly prepared racemic **1b**, which was dissolved at 0.66 mg/mL in acetone/ether/tetrahydrofuran (18.5/11/1). After butanol extraction, deoxyribonucleotide adducts were recovered from Sep-Pak Cartridges hydrolyzed with alkaline phosphatase to nucleosides and again recovered from Sep-Pak Cartridges in similar procedures to those described for DNA reactions in the previous text.

(3) The term bay region dihydrodiol epoxide is used to designate a vicinal dihydrodiol epoxide in which the epoxide ring is adjacent to a bay region, *i.e.*, that region between C_1 and C_{12} of benz[*a*]anthracene. A *syn*-dihydrodiol epoxide has the epoxide oxygen and the benzylic hydroxyl group cis to one another, whereas in an *anti*-dihydrodiol epoxide, these groups are trans.

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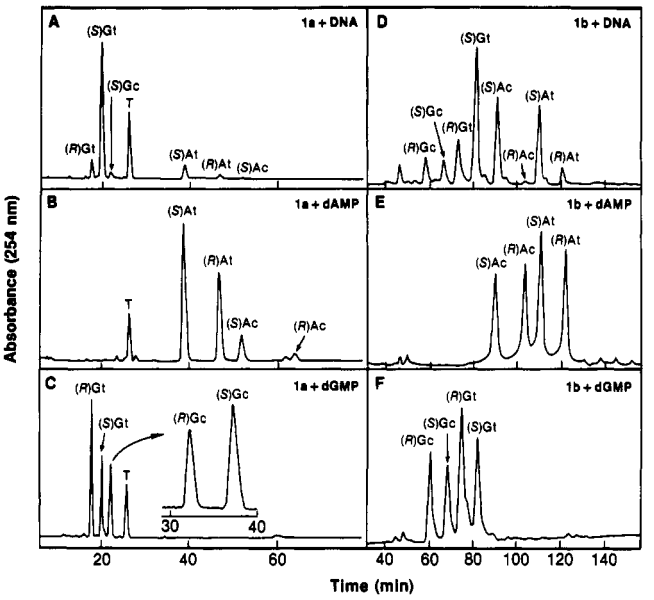

Figure 1. High-pressure liquid chromatography of deoxyribonucleoside adducts derived from reactions of **1a** and **1b** with DNA and purine nucleotides. The other abbreviated labels are explained in Scheme I. T indicates the position of elution of the *trans*-tetrol from **1a**.

Adduct Separations. Deoxyribonucleoside adducts from reactions with DNA or nucleotides were separated chromatographically on an Altex Ultrasphere ODS column (5 μm, 4.6 × 250 mm) using isocratic elution with 22% acetonitrile in water for the adducts from **1a** and using the methanol/water gradient system described earlier[20] for the adducts from **1b**. Products from the nucleotide reactions were pooled from several chromatographic runs for further characterizations. In the case of the deoxyguanosine adducts from **1a**, one recovered peak was rechromatographed in 16% tetrahydrofuran in water to resolve it into its two constituents. After recording CD spectra for these isolated adducts, they were reduced to dryness, acetylated with acetic anhydride, and repurified on a normal-phase column (Zorbax Sil, 4.6 × 250 mm, 5 μm) eluted isocratically with dichloromethane/ethyl acetate/methanol (160/30/1) for deoxyadenosine adducts from **1a**, (160/30/3) for deoxyadenosine adducts from **1b**, (160/30/5) for deoxyguanosine adducts from **1a**, and (160/30/10) for deoxyguanosine adducts from **1b**. Tetrols derived from cis opening of the epoxide ring (cis tetrols) from 7MeBA and DMBA were obtained by osmium tetroxide oxidations of the appropriate 3,4-dihydrodiols,[21] which were prepared as described.[17,18] Tetrols derived from trans opening of the epoxide ring (trans tetrols) were isolated from the organic extracts of the dihydrodiol epoxide reactions with nucleotides.

Results and Discussion

Reactions of the racemic dihydrodiol epoxides **1a** and **1b** (Scheme I) with calf thymus DNA *in vitro*, followed by enzymic hydrolysis to nucleosides and chromatographic separation of the hydrocarbon-containing products showed the presence of several products in each case (Figure 1A and 1D). Comparison of the retention times and ultraviolet spectra for these adducts with those obtained for nucleoside adducts derived from reactions with the mononucleotides deoxyadenosine 5′-phosphate (Figure 1B and 1E) and deoxyguanosine 5′-phosphate (Figure 1C and 1F) allowed the nucleoside of origin of each of the DNA adducts to be readily established. These assignments and the overall structural characterizations resulting from the studies described herein are summarized in the abbreviated

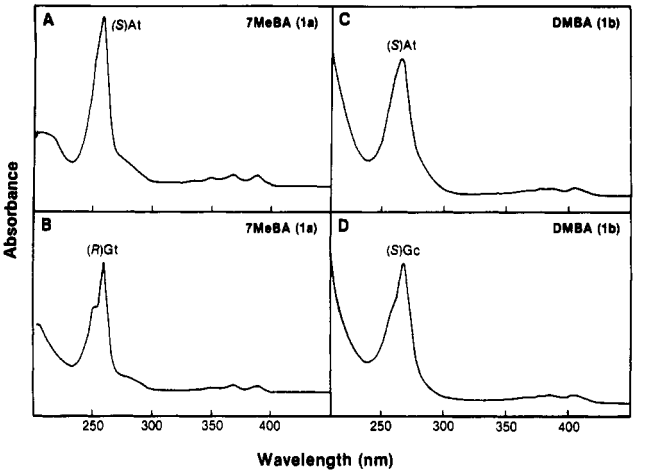

Figure 2. Ultraviolet spectra of deoxyribonucleoside adducts derived from **1a** and **1b**. See Scheme I for explanation of labels.

labels given to each adduct in Figure 1. As summarized in Scheme I, these labels indicate the absolute stereochemistry at C_1 of the hydrocarbon residue (*R* or *S*), the nucleoside attached through its amino group to C_1 (*i.e.*, deoxyguanosine (G) or deoxyadenosine (A)), and the relationship between the purine nucleoside and the hydroxyl group on C_2 (*i.e.*, cis (c) or trans (t)). The ultraviolet absorption spectra (Figure 2) allow deoxyadenosine adducts to be distinguished from deoxyguanosine adducts for both **1a** and **1b** reactions, but for each dihydrodiol epoxide, the spectra of all the deoxyadenosine adducts were similar as were those of the deoxyguanosine adducts. Deoxyadenosine and deoxyguanosine adducts derived from **1a** (λ_{max} 259 and 260 nm, respectively) absorbed at shorter wavelengths than those derived from **1b** (λ_{max} 266 and 268 nm, respectively) but the general shape of the spectra for the two hydrocarbons was similar, as expected.

Once the relationships between the products obtained from DNA and nucleotide reactions were established by chromatography and ultraviolet absorbance spectroscopy, large amounts of the nucleoside adducts derived from the nucleotide reactions were collected by pooling material from several chromatographic runs. These materials were then used to collect CD spectra (Figure 3) that enabled the stereochemical relationships between the products from each dihydrodiol epoxide to be established. It has been found in previous work[7-11] that the CD spectra of the four nucleoside adducts derived from the two enantiomers of a given diastereomer of a dihydrodiol epoxide (*i.e.*, the cis and trans products from each enantiomer) constitute two pairs of mirror images. One mirror image pair represents the two cis products and the other the two trans products. It can be seen in Figure 3 that the same situation was obtained for the adducts derived from **1a** and **1b**. Although the labels indicate which spectra derive from the cis and trans products, it must be noted that this information is not derived from the CD spectra but from the NMR data that follows.

After collection of CD spectra, adducts and tetrols were acetylated for ^1H NMR studies, since in previous work acetone solutions of peracetates had yielded excellent spectra.[7-11] Extensive analysis of the trans tetrol tetraacetate from 7-methylbenz[*a*]anthracene allowed all of the protons to be definitively assigned. The coupling patterns allowed the aromatic resonances to be assigned (Figure 4). Thus, the singlet at 8.35 ppm is the proton on C_{12}, the two mutually coupled resonances at 8.52 and 7.40 ppm are the protons on C_6 and C_5, respectively, and the four mutually coupled aromatic resonances left are the 8–11 protons.

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Table I. ^{1}H NMR Data for Acetates of Deoxyribonucleoside Adducts and of Tetrols from Dihydrodiol Epoxides 1a and 1b

compd (as the acetate)	methine hydrogens								
	N-H		C_1-H		C_2-H		C_3-H		C_4-H
1a *trans*-tetrol			6.80	$J_{1,2}$ = 3.7	5.78	$J_{2,3}$ = 2.4	5.61	$J_{3,4}$ = 8.9	6.55
1a (*S*)At	7.07		6.62	$J_{1,2}$ = ~3	5.98	$J_{2,3}$ = 2.4	5.96	$J_{3,4}$ = 9.03	6.55
1a (*R*)At	7.04		6.63	$J_{1,2}$ = ~2–3	~5.97	$J_{2,3}$ = ~2.5	~5.96	$J_{3,4}$ = 8.84	6.54
1a (*R*)Gt	7.25	$J_{NH,1}$ = 7.0	6.16	$J_{1,2}$ = 2.8	6.05	$J_{2,3}$ = 2.4	5.77	$J_{3,4}$ = 8.9	6.55
1a (*S*)Gt	7.29		6.15	$J_{1,2}$ = 2.5	6.10	$J_{2,3}$ = 2–2.5	5.75	$J_{3,4}$ = 8.9	6.53
1a *cis*-tetrol			7.18	J = 4.8	5.78	J = 2.4	5.69	J = 3.6	6.30
1a (*S*)Ac	6.63	$J_{NH,1}$ = 10.5	7.03	$J_{1,2}$ = 5.4	5.81	$J_{2,3}$ = 2.2	5.70	$J_{3,4}$ = 2.5	6.34
1a (*R*)Ac									
1a (*R*)Gc	6.26	$J_{NH,1}$ = 9.5	6.74	$J_{1,2}$ = 5.1	5.84	$J_{2,3}$ = 2.4	5.65	$J_{3,4}$ = 3.2	6.30
1a (*S*)Gc	6.35	$J_{NH,1}$ = 9.3	6.83	$J_{1,2}$ = 4.9	5.82	$J_{2,3}$ = 2.3	5.64	$J_{3,4}$ = 3.5	6.33
1b *trans*-tetrol			7.01	$J_{1,2}$ = 4.76	5.84	$J_{2,3}$ = 2.66	5.63	$J_{3,4}$ = 8.24	6.51
1b (*S*)At	7.16		7.01		6.11		5.84	$J_{3,4}$ = 8.79	6.45
1b (*R*)At	7.17		7.03		6.11		5.83	$J_{3,4}$ = 8.79	6.45
1b (*R*)Gt	5.79		6.48	$J_{1,2}$ = 4.03	6.24	$J_{2,3}$ = 2.56	5.69	$J_{3,4}$ = 8.42	6.50
1b (*S*)Gt	6.37		6.52	$J_{1,2}$ = 4.2	6.20	$J_{2,3}$ = 2.56	5.69	$J_{3,4}$ = 8.09	6.49
1b *cis*-tetrol			7.31	$J_{1,2}$ = 3.23	5.95	$J_{2,3}$ = 2.56	5.70	$J_{3,4}$ = 8.06	6.60
1b (*S*)Ac	6.30	$J_{NH,1}$ = 8.6	7.24	$J_{1,2}$ = 4.21	6.17	$J_{2,3}$ = 2.20	5.76	$J_{3,4}$ = 6.77	6.57
1b (*R*)Ac	6.28		7.24	$J_{1,2}$ = 4.16	6.17	$J_{2,3}$ = 2.20	5.76	$J_{3,4}$ = 6.96	6.57
1b (*R*)Gc	5.84	$J_{NH,1}$ = 7.23	6.73	$J_{1,2}$ = 4.10	6.37	$J_{2,3}$ = 1.92	5.68	$J_{3,4}$ = 8.88	6.63
1b (*S*)Gc	5.85	$J_{NH,1}$ = 7.40	6.97	$J_{1,2}$ = 4.1	6.23	$J_{2,3}$ = 1.7	5.72	$J_{3,4}$ = 8.90	6.61

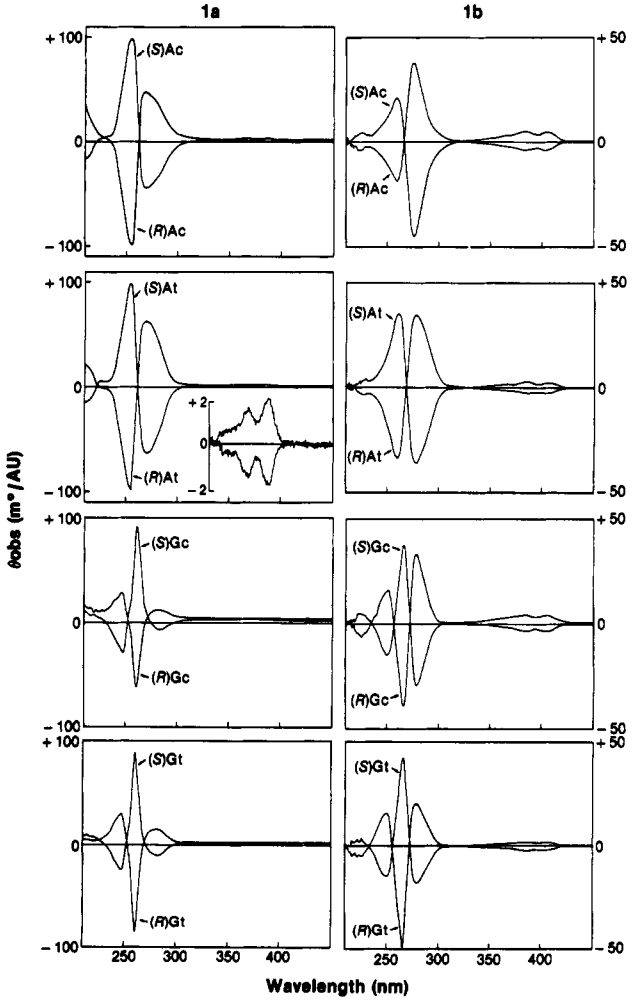

Figure 3. Circular dichroism spectra of deoxyribonucleoside adducts derived from 1a and 1b. See Scheme I for explanation of labels.

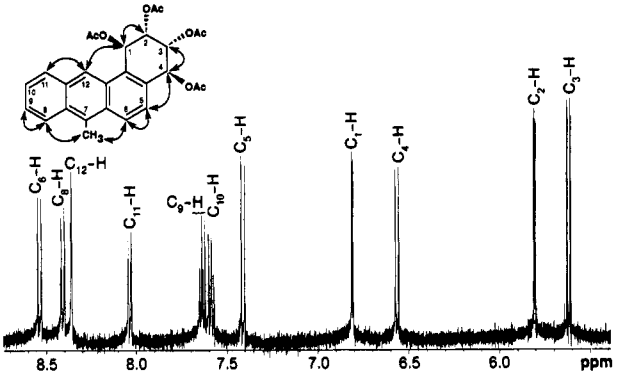

Figure 4. Downfield region of the NMR spectrum of the tetraacetate of 1a tetrol. The insert shows NOE connections between protons.

These, and the protons in the tetrol ring, were assigned with the NOE results summarized in Figure 4. One of the doublets at either 6.55 or 6.79 ppm must be the proton on C_1 and the NOE connection between the C_{12} proton and the 6.79 resonance indicates that this latter resonance corresponds to the proton on C_1. This is confirmed by the NOE connection between the 6.55 ppm resonance and the proton on C_5, indicating that the 6.55 ppm resonance is due to the proton on C_4. The protons on C_2 and C_3 are then readily assignable from their coupling to the protons on C_1 and C_4, respectively. The relative downfield shift of the proton on C_1 is consistent with its location in a bay region and the coupling pattern of the protons in the tetrol ring (Table I) (*i.e.*, $J_{3,4}$, $J_{2,3}$, and $J_{1,2}$ are 8.9, 2.4, and 3.7 Hz, respectively) is very similar to that previously reported for the trans tetraol tetraacetate from the *anti* diastereomer of benzo[*a*]pyrene dihydrodiol epoxide.[8,20] These coupling constants indicate that the protons on C_3 and C_4 are pseudodiaxial in the trans tetrol. A less detailed analysis of the cis tetraol was undertaken because of the limited amount that was obtained. The aromatic protons were very similar to those of the trans tetrol but the methine protons were quite different with coupling constants (Table I) indicating a pseudodiequatorial conformation for the protons on C_3 and C_4. Thus, the conformations of cis and trans tetrols from 7MeBA are quite different as was found for benzo[*a*]pyrene.[7,22] Seven purine deoxyribonucleoside adducts were obtained in quantities sufficient to allow NMR analysis (Table I, Figure 5). It can be seen that the trans adducts all displayed characteristics similar to those of the trans tetrol and the cis adducts resembled the cis tetrol. In several cases, a clear NH signal was detected and D_2O exchange studies in these cases clearly established the linkage between C_1 and the purine amino group and confirmed the assignment of the

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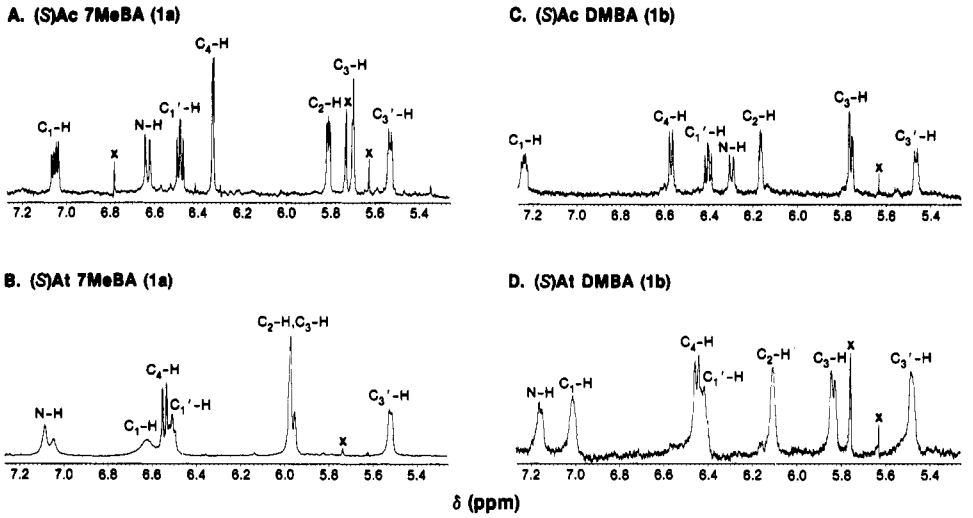

Figure 5. Downfield region of the NMR spectra four hydrocarbon–deoxyribonucleoside adduct peracetates. Structures are indicated in Scheme I, and X indicates signals from impurities in the samples.

proton on C_1. In all cis–trans pairs, the proton on C_1 is further downfield in the cis product than in the corresponding trans product, extending the empirical relationship noted earlier;[8] this relationship has been noted in related model systems.[23]

Spectral data from analysis of the two tetrols and eight adducts obtained from **1b** (the DMBA dihydrodiol epoxide) are also presented in Table I, and two adduct spectra are illustrated in Figure 5. Both tetrol tetraacetates gave very clear spectra, and the coupling constants allowed the linkages in the tetrol ring to be readily established. However, other than the chemical shifts, there was no clear way of assigning the protons on C_1 and C_4. This was done by consideration of the data for the adducts where coupling between the N–H and C_1–H permitted unambiguous assignment of C_1–H. These DMBA derivatives are similar to the corresponding 7MeBA derivatives in the case of the trans products, but notable differences are seen when the cis products formed from these two hydrocarbons are compared. The coupling constants indicate that C_4–H and C_3–H in cis products from **1a** are pseudodiequatorial in contrast with the pseudodiaxial conformation found in the cis products from **1b**.

The conformation adopted by the partially unsaturated 1,2,3,4-ring in all the various adducts can be estimated from the NMR coupling constants presented in Table I. For trans adducts, coupling constants are fairly similar in both the **1a** and **1b** series and they suggest that the hydrogens on C_3 and C_4 must have a very large dihedral angle, *i.e.*, they are pseudodiaxial in both the **1a** and **1b** series. Since $J_{2,3}$ is small in both series, the hydrogen on C_2 is presumably pseudoequatorial such that the dihedral angle between C_2–H and C_3–H approaches 90°. The conformation shown in Chart Ia with the purine in a pseudodiaxial position accounts for the NMR findings for trans adducts in both the **1a** and **1b** series although small differences in coupling constants for the two series indicate that there must be some minor conformational differences arising from different degrees of distortion from the conformation in Chart Ia. It should be emphasized that with a double bond in the 1,2,3,4-ring, the substituents are only *pseudo*axial or *pseudo*equatorial, and particularly in the case of substituents on C_1, model building suggests that they may lie close to the midway point between axial and equatorial positions.

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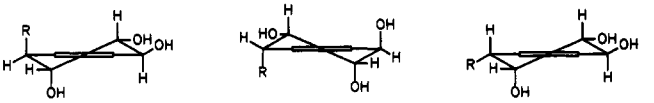
Chart I. Conformations of Different Types of Nucleoside Adducts: (a) Trans Adducts from 1a and 1b; (b) Cis Adducts from 1a; (c) Cis Adducts from 1b



(a) (b) (c)

In contrast to the similarities in the trans adducts, the conformations of the cis adducts from the **1a** and **1b** series are clearly different. For example, $J_{3,4}$ is ~3 Hz for deoxyguanosine adducts in the **1a** derivatives, whereas it is ~9 Hz for the corresponding **1b** derivatives. Thus, in the **1a** series the hydrogens on C_3 and C_4 are pseudodiequatorial, as illustrated in Chart Ib whereas, in the **1b** series, they are pseudodiaxial (Chart Ic). In the **1a** series, the hydrogen on C_2 must be pseudoaxial to yield a dihedral angle for C_3–H and C_2–H approaching 90° and the small coupling constant observed. The larger coupling for $J_{1,2}$ suggests a smaller dihedral angle for C_1–H and C_2–H, and this is achieved when the purine substituent is pseudoaxial, as shown in Chart Ib.

In the cis adducts in the **1b** series, placing the purine in a pseudoaxial position to minimize steric interactions with the bay region methyl group leads to the eclipsing of the protons on C_2 and C_3, which is inconsistent with the very small $J_{2,3}$ found experimentally. Thus, the purine must be more pseudoequatorial, giving a dihedral angle for C_2–H and C_3–H approaching 90°, as illustrated in Chart Ic.[24] Again, it should be noted that the purine is not truly equatorial, and thus, steric hindrance between it and the bay region methyl group is somewhat relieved. Again, there is an element of choice in defining the orientation of the purine in these structures as pseudoaxial or pseudoequatorial since it resides between the classical axial and equatorial positions.

Molecular modeling, which was used to help visualize the various adduct structures, was reasonably consistent with the experimental findings. For both cis and trans products the structures from the **1a** and **1b** series were

(24) A reviewer has suggested that the cis adducts in the **1b** series occupy a distorted boatlike conformation rather than the chairlike conformation of Chart Ic. This boatlike conformation eclipses C_2–H and C_3–H, which is inconsistent with their coupling constant, and distortion of this structure to provide a greater dihedral angle for these protons distorts the structure toward the chairlike conformer we have suggested.

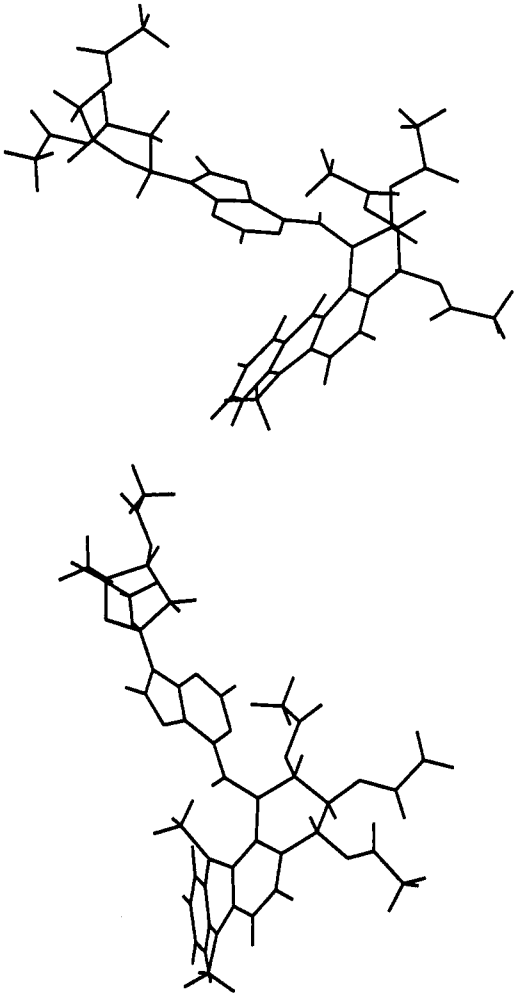


Figure 6. Three-dimensional representation of *cis*-deoxyadenosine adducts from **1a** (upper) and **1b** (lower) obtained from computer models of these structures constrained by the experimental data. The models predict angles for H_2–C_2–C_1–N^6, H_2–C_1–C_2–H_2, H_2–C_2–C_3–H_3, and H_3–C_3–C_4–H_4 of 170, 50, −60, and −70° for the **1a** adduct and 90, −30, 60, and −180° for the **1b** adduct.

quite different with respect to the mutual orientation of the purine ring and the aromatic residue, as shown in Figure 6 for the *cis*-deoxyadenosine adducts. The different angles between the two chromophores in **1a** and **1b** are consistent with the different intensities of CD signals (Figure 3). Additionally, it can be seen that the DMBA residue is more twisted than the 7MeBA residue in these structures.

It was possible to assign absolute stereochemistry to the **1b** products because assignments have previously been made by identifying products formed in cell cultures from optically active dihydrodiols.[16] With each chromatographic analysis shown in Figure 1D–F, a sample of radioactive deoxyribonucleoside adducts from the DNA of cells exposed to [^{3}H]DMBA was included. This allowed the identification of the peaks labeled (*S*)Gt and (*S*)At as the major products formed from the (4*R*,3*S*)-dihydrodiol (2*S*,1*R*)-epoxide *in vivo*. Since the NMR studies, discussed previously, indicate that these products are trans about the 1,2-bond, the absolute stereochemistry at C_1 must be *S* as indicated in the abbreviated label for these products. Since the analogous adducts from the (4*S*,3*R*)-dihydrodiol (2*R*,1*S*)-epoxide will have CD spectra that are exact mirror images of those from the other enantiomer, the absolute stereochemistry of the products labeled (*R*)At and (*R*)Gt was then assigned. Knowing that the *cis*-deoxyguanosine

Table II. Distribution of Adducts Obtained from Reaction with DNA or Purine Nucleotides

dihydrodiol epoxide	adducts distribution (%)							
	DNA				nucleotides			
	dGuo		dAdo		dGuo		dAdo	
	cis	trans	cis	trans	cis	trans	cis	trans
1a (*R,S,S,R*)	6	66	0	14	12	29	4	48
1a (*S,R,R,S*)	0	9	1	4	16	43	12	36
1b (*R,S,S,R*)	7	33	1	16	20	24	21	32
1b (*S,R,R,S*)	7	14	19	3	19	37	25	22

product from the (4*R*,3*S*)-dihydrodiol (2*S*,1*R*)-epoxide will have a CD spectrum that is almost a mirror image of the trans product[7-11] allowed absolute stereochemistry to be assigned to the product labeled (*R*)Gc, and in this fashion, each of the DMBA adducts were assigned absolute stereochemistry as shown in Scheme I.

Although we had no direct means of assigning stereochemistry to the 7MeBA adducts, the circular dichroism spectra showed such great similarities to those of the DMBA series that we felt comfortable in making tentative assignments on this basis (Figure 3). The assignments from the CD spectra are reasonable because this indicates that, as has been found with other hydrocarbon dihydrodiol epoxides,[1,5-10,25] the major deoxyguanosine and deoxyadenosine adducts formed with the racemic *anti*-dihydrodiol epoxides upon reaction with DNA are trans products from the (4*R*,3*S*)-dihydrodiol (2*S*,1*R*)-epoxide.

The product distributions for each dihydrodiol epoxide examined are summarized in Table II. In reactions with DNA, the (4*R*,3*S*)-dihydrodiol (2*S*,1*R*)-epoxide from both hydrocarbons reacts preferentially to form a trans deoxyguanosine adduct. However, this reaction is more selective for **1a** where the trans deoxyguanosine adduct represents a larger fraction of total products than for **1b**. Also for **1a**, the *R,S,S,R* enantiomer accounts for 86% of the DNA products arising from the racemate, whereas for **1b**, this enantiomer accounts for only 57% of products. The lower selectivity in the reactions of the bay region substituted hydrocarbon (**1b**) with DNA is also evident from the fact that, whereas 39% of its DNA products arise from reaction with deoxyadenosine, only 19% of the DNA products from **1a** arise from such a reaction. Additionally, with both DNA and nucleotides, the strong preference of **1a** for trans product formation is not matched by **1b**, which gives substantial yields of cis products. Thus, the presence of a bay region methyl group in **1b** leads to a less selective reaction with DNA that results in a more even distribution of the hydrocarbon over both adenine and guanine residues than is found for **1a** and a greater contribution from cis as well as trans products.

Conclusions

The present findings extend our knowledge of the structures of hydrocarbon carcinogen–DNA adducts considerably and present for the first time a direct comparison of the spectral properties of adducts formed from a bay region substituted and the corresponding unsubstituted hydrocarbon dihydrodiol epoxide. As has been found for other hydrocarbons, the principal sites of reaction are the amino groups of the purine bases in DNA.[6-11,25] Despite some tentative identifications of O^6- or 7-substituted deoxyguanosines with benzo[*a*]pyrene dihydrodiol epoxides,[26]

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(26) Osborne, M. R.; Jacobs, S.; Harvey, R. G.; Brookes, P. *Carcinogenesis* **1981**, *2*, 553.

amino group substituted purine and pyrimidine nucleoside adducts are the only adducts for which reasonable spectroscopic characterizations have been achieved. Model studies of guanosine aralkylation with benzylating agents[27] and with styrene oxide[28] have indicated that substrates reacting through ionic intermediates, in which charge at the reaction center is effectively delocalized, preferentially react at the amino group, and the reaction of the hydrocarbon metabolites studied herein are certainly consistent with this concept.[29]

The findings presented herein are similar to previous studies for other hydrocarbons in terms of the conformational effects of a bay region substituent. Thus, the NMR findings indicating a pseudoaxial conformation for the hydrogens on C_3 and C_4 in cis adducts from the bay region substituted **1b** is consistent with the reported findings for the corresponding benzo[*c*]phenanthrene[8] and 5-methylchrysene[10] derivatives. Similarly, the pseudoequatorial conformation for these hydrogens in cis adducts from **1a** is consistent with observations for other hydrocarbons unsubstituted in the bay region, *i.e.*, benzo[*a*]pyrene[7] and dibenz[*a,j*]anthracene.[9] In other respects, however, there are considerable differences between the hydrocarbons reported on herein and those reported previously. Of particular interest is the ratio of cis and trans adduct formation in reactions with nucleotides. Whereas we previously found a roughly 1:1 distribution of cis and trans adducts for benzo[*a*]pyrene dihydrodiol epoxide,[7] the present work shows that another hydrocarbon unsubstituted in the bay region, **1a**, preferentially yields trans adducts. The reasons for this difference are not well defined, but it is conceivable that the more extensive aromatic system in the benzo[*a*]pyrene dihydrodiol epoxide might favor a more ionic reaction mechanism than for the benz[*a*]anthracene derivative. Curiously, the addition of the 12-methyl group to **1a** to give **1b** dramatically changes the cis/trans adduct ratio to something close to the 1:1 ratio seen for the benzo[*a*]pyrene derivatives. This, however, is substantially different from the ratios seen for other bay region substituted hydrocarbons since trans adducts predominate and cis adducts represent only minor products in reactions of both 5-methylchrysene dihydrodiol epoxide[10] and benzo[*c*]phenanthrene dihydrodiol epoxide[8] with nucleotides.

The accumulating data on hydrocarbon dihydrodiol epoxide–DNA and –nucleotide reactions raise some interesting points. In terms of the conformation of the partially saturated ring in the adducts, estimated from NMR data, the six hydrocarbons for which there are now extensive spectral analyses of adducts would be classified into two groups: bay region unsubstituted hydrocarbons (benzo[*a*]pyrene, 7-methylbenz[*a*]anthracene, and dibenz[*a,j*]anthracene) for which cis adducts adopt the conformation illustrated in Chart Ib and bay region substituted hydrocarbons (benzo[*c*]phenanthrene, 5-methylchrysene, and 7,12-dimethylbenz[*a*]anthracene) for which cis adducts adopt the conformation illustrated in Chart Ic. In terms of the extent of reaction with deoxyadenosine residues in DNA, two different groupings are apparent: extensive reaction with deoxyadenosine residues in DNA is associated with large out-of-plane distortions in the parent hydrocarbons (*i.e.*, benzo[*c*]phenanthrene[12,30] and 7,12-dimethylbenz[*a*]anthracene[31,32]), whereas less extensive deoxyadenosine reaction is observed in structures with less distortion (*i.e.*, benzo[*a*]pyrene and dibenz[*a,j*]anthracene) even though they may be similarly substituted in the bay region (*i.e.*, 5-methylchrysene). Thirdly, in terms of cis/trans adduct ratios in reactions with nucleotides, the studied hydrocarbon dihydrodiol epoxides fall into yet another grouping wherein something approaching a 1:1 ratio is found for benzo[*a*]pyrene[7] and 7,12-dimethylbenz[*a*]anthracene derivatives, whereas cis adducts are relatively minor components for 5-methylchrysene,[10] benzo[*c*]phenanthrene,[8,12] and 7-methylbenz[*a*]anthracene derivatives. These different groupings of hydrocarbons should be explicable when the chemistry of these interesting dihydrodiol epoxides is fully understood.

In this report, differences between the bay region substituted **1b** and unsubstituted **1a** metabolites are of particular interest because hydrocarbons with bay region methyl groups tend to be more potent carcinogens than the unsubstituted homologues.[13] Unfortunately, the instability of current preparations of **1b**[18] precluded measurements of overall product yields. However, substantial differences in product distributions for **1a** and **1b** were noted. In this regard, the bay region methyl substituent in **1b** decreased selectivity with respect to **1a** with the result that a greater fraction of total reaction with DNA occurs with deoxyadenosine residues for **1b** than for **1a**. Previous studies have also suggested an association between carcinogenic potency and reactivity towards deoxyadenosine residues.[8,11,12,31] This association is supported by findings in Balmain's laboratory that showed AT → TA transversions at the second position of codon 61 in activated *ras* oncogenes extracted from DMBA-induced tumors.[33]

The second major difference in adduct formation from **1a** and **1b** lies in the conformations of the adducts. NMR studies indicate that the trans products from either **1a** or **1b** adopt a conformation with the purine nucleoside being pseudoaxial and the hydrogens on C_3 and C_4 being pseudodiaxial (Chart Ia). In contrast, the C_3 and C_4 hydrogens in the cis adducts from **1a** are pseudodiequatorial with the purine nucleoside in the bay region being disposed pseudoaxially (Chart Ib). The cis products from **1b** are quite different and have the purine nucleoside in the apparently more hindered pseudoequatorial conformation in the bay region. These conformational differences also emerge from modeling studies (Figure 6). Though we describe the purine as pseudoequatorial, models indicate that it lies between truly axial and equatorial positions. The C_3 and C_4 hydrogens are pseudodiaxial (Chart Ic) in this case. This pronounced difference in conformation of cis adducts for the two hydrocarbons could contribute to the different carcinogenic potencies of the parent compounds.

Although the NMR data suggest that the conformations of the 1,2,3,4-rings in the trans products from both **1a** and **1b** are similar, molecular modeling studies indicated that some major conformational differences do exist. Thus, the greater carcinogenic potency of DMBA with respect to

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7MeBA could arise either from the conformational differences of the adducts formed on DNA, from the lesser selectivity of the dihydrodiol epoxides of DMBA versus 7MeBA in reactions with DNA, from differences in extent of DNA modification, from difficulties in repairing DMBA-DNA damage,[34] or any combination of these differences associated with methyl substitution in the bay region.

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Registry No. 1a, 133645-02-0; 1a + (*R*)Gt, 133551-46-9; 1a + (*S*)Gt, 133646-13-6; 1a + (*S*)Gc, 133645-03-1; 1a + (*S*)At, 133551-47-0; 1a + (*R*)At, 133645-04-2; 1a + (*S*)Ac, 133645-05-3; 1a + (R)Ac, 133645-06-4; 1a + (*R*)Gc, 133645-07-5; 1b, 130856-45-0; 1b + (*R*)Gc, 133551-48-1; 1b + (*S*) Gc, 133645-08-6; 1b + (*R*)Gt, 133645-09-7; 1b + (*S*)Gt, 133645-10-0; 1b + (*S*)Ac, 133551-49-2; 1b + (*R*)Ac, 133645-11-1; 1b + (*S*)At, 133694-83-4; 1b + (*R*)At, 133645-12-2; dAMP, 653-63-4; dGMP, 902-04-5.

Structure-Activity Relationship in the Quenching Reaction of Singlet Oxygen by Tocopherol (Vitamin E) Derivatives and Related Phenols. Finding of Linear Correlation between the Rates of Quenching of Singlet Oxygen and Scavenging of Peroxyl and Phenoxyl Radicals in Solution

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The rate of quenching of $^1O_2$ by 17 kinds of tocopherol derivatives, including α-, β-, γ-, and δ-tocopherols, and five structurally related phenols has been measured spectrophotometrically in ethanol at 35 °C. The result indicates that the overall rate constants, k_Q ($k_Q = k_q + k_r$, physical quenching + chemical reaction), increase as the total electron-donating capacity of the alkyl substituents on the aromatic ring increases. The log of the rate constants, k_Q, was found to correlate with their half-peak oxidation potentials, $E_{P/2}$; the tocopherols that have smaller $E_{P/2}$ values show higher reactivities. Tocopherols 11 and 12 with a five-membered heterocyclic ring were found to be 1.73 and 1.21 times more active than α-tocopherol, respectively, which has the highest antioxidant activity among natural tocopherols and related phenols. Two benzodipyran derivatives 16 and 17 having no OH group were also found to be 1.63 and 1.33 times more active than the α-tocopherol. The quenching rates, k_Q, observed were found to be related linearly to the rates k_1 and k_3 of scavenging of peroxyl and phenoxyl radicals by these tocopherols, respectively, reported previously by Burton et al. and by Mukai et al., except for the benzodipyran derivatives. The result indicates that the relative reactivities, that is, relative antioxidant activities of phenolic antioxidants in homogeneous solution, do not depend on singlet oxygen ($^1O_2$), peroxyl radical (LOO•), and substituted phenoxyl radical (PhO•) reacted. Further, the result indicates that the properties of the transition states in the singlet oxygen quenching and free radical scavenging reactions by tocopherol are similar, suggesting a charge-transfer intermediate.

Introduction

Vitamin E compounds (α-, β-, γ-, and δ-tocopherols) are well-known as scavengers of active free radicals (LOO•, LO•, and HO•) generated in biological systems.[1,2] Recently, Burton et al.[3] have reported absolute second-order rate constants, k_1, for the reaction of α-, β-, γ-, and δ-tocopherols and related phenols with poly(styrylperoxyl)-peroxyl radicals using the inhibited autoxidation of styrene method (reaction 1).

$$\text{LOO•} + \text{Toc} \xrightarrow{k_1} \text{LOOH} + \text{Toc•} \quad (1)$$

It was observed that the second-order rate constants, k_1, of tocopherols decrease in the order of $\alpha > \beta \approx \gamma > \delta$-tocopherol. We reported that the absolute reactivities of tocopherols to LOO• increase as the total electron-donating capacity of the alkyl substituents at the aromatic ring increases.[4,5] For the tocopherols log k_1 was found to correlate roughly with the sum of Brown's σ^+ constants ($\Sigma\sigma^+$). Further, the log of the second-order rate constants, k_1, obtained for the tocopherols was found to correlate with their half-peak oxidation potentials, $E_{P/2}$; the tocopherols which have smaller $E_{P/2}$ values show higher reactivities.

On the other hand, tocopherols can also act as an efficient scavenger of singlet oxygen ($^1O_2$).[6-8] It was shown that α-tocopherol scavenges $^1O_2$ by a combination of physical quenching (k_q) and chemical reaction (chemical quenching, k_r). Because $k_q \gg k_r$, the quenching process is almost entirely "physical"—that is, α-tocopherol deactivates ~120 $^1O_2$ molecules before being destroyed by chemical reaction[6] (reaction 2).

$$^1O_2 + \text{Toc} \xrightarrow{k_Q} \text{physical quenching} + \text{chemical reaction} \quad (2)$$

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