then calculated (intercept/slope).

2. **Thymidylate Kinase.** Enzyme activity was measured essentially as described by Lee and Cheng[15] using [$^3$H]TMP (24 $\mu$M) and ATP (1.5 mM) as cosubstrates. A dialyzed extract of blast cells received from a patient with acute myeloid leukemia was used as the enzyme source. The data were fitted by a nonlinear least-squares regression program based on the algorithm described by Jennrich and Sampson.[16] For determination of $IC_{50}$ values, the data were fitted to the equation (response) = $a + b$ ln [inhibitor] with an error weighting of $1/(y + y)^2$. [17,18]

3. **Ribonucleotide Reductase.** A crude extract was prepared from L1210 cells and adjusted to pH 5.2 with 1 M acetic acid. The precipitate was collected, dissolved in 50 mM Tris·HCl, pH 8.0, and used as the enzyme source. CDP reductase activity was measured as described by Cory and Mansell.[19] The data were analyzed in the same way as those for thymidylate kinase.

**Acknowledgment.** This investigation was supported by grants from the Cancer Research Campaign and the Medical Research Council to The Institute of Cancer Research. We thank the University of London Intercollegiate Research Service at King's College for the NMR spectra.

(19) Cory, J. G.; Mansell, M. M. *Cancer. Res.* **1975**, *35*, 2327.

# Synthesis of the Tumorigenic 3,4-Dihydrodiol Metabolites of Dibenz[*a*,*j*]anthracene and 7,14-Dimethyldibenz[*a*,*j*]anthracene

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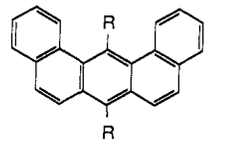
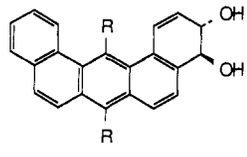
Syntheses are described of the *trans*-3,4-dihydrodiol derivatives (**2a** and **2b**) of dibenz[*a*,*j*]anthracene and 7,14-dimethyldibenz[*a*,*j*]anthracene (**1a** and **1b**), implicated as their *proximate* carcinogenic metabolites. Conversion of **2a** to the bay region *anti*-diol epoxide derivative **3a**, its putative *ultimate* carcinogenic metabolite, is also reported. The related diol epoxide derivative of **2b** could not be prepared due to its chemical instability. Tumorigenicity assays confirm that **1b** and **2b** are potent carcinogens on mouse skin, while **1a** and **2a** are only relatively weakly active. The diol epoxide **3a** exhibited significantly higher tumorigenicity than its dihydrodiol precursor **2a**. These findings are consistent with the hypothesis that the bay region diol epoxide metabolites are the active carcinogenic forms of these hydrocarbons. They also support the generalization that methyl substitution in bay regions enhances the carcinogenic activity of polycyclic aromatic hydrocarbons.

Introduction of methyl groups into the nonbenzo bay region positions of polycyclic aromatic hydrocarbons often markedly enhances their potency as carcinogens.[1,2] One of the most dramatic examples of this "bay region methyl effect" is 7,14-dimethyldibenz[*a*,*j*]anthracene (**1b**).[3] While the parent hydrocarbon dibenz[*a*,*j*]anthracene (**1a**) exhibits only weak borderline activity as a carcinogen on mouse skin, the dimethyl analogue is a potent carcinogen, its activity rivalling that of the highly potent 7,12-dimethylbenz[*a*]anthracene.[2]

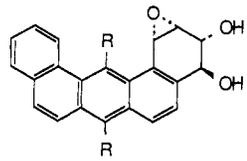
It has been established that polycyclic hydrocarbons require metabolic activation to express their biological potential, and the principal active metabolites have been identified as the bay region diol epoxides.[4] As part of a program to elucidate the molecular basis of the "bay region methyl effect", we required the bay region *anti*-diol epoxides of **1a** and **1b** (**3a** and **3b**) and their 3,4-dihydrodiol precursors (**2a** and **2b**). We now report the synthesis of *trans*-3,4-dihydroxy-3,4-dihydrodibenz[*a*,*j*]anthracene (**2a**) *trans*-3,4-dihydroxy-3,4-dihydro-7,14-dimethyldibenz[*a*,*j*]anthracene (**2b**), and *trans*-3,4-dihydroxy-*anti*-1,2-epoxy-1,2,3,4-tetrahydrodibenz[*a*,*j*]anthracene (**3a**).




**1**




**2**




**3**

**a: R = H; b: R = $CH_3$**

## Results and Discussion

The synthetic approach to the dihydrodiols **2a** and **2b** is based upon the general method reported earlier for the conversion of phenols to dihydrodiols.[5,6] 3-Hydroxydibenz[*a*,*j*]anthracene (**9b**) required as the starting compound for the synthesis of **2a** was itself synthesized via the sequence in Scheme I. Metalation of the appropriate *N*,*N*-diethylarylamide with *sec*-butyllithium in ether by the method of Beak[7] furnished *N*,*N*-diethyl-2-lithio-6-methoxy-1-naphthamide (**5a**). Condensation of **5a** with

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(1) Hecht, S. S.; Amin, S.; Melikian, A. A.; LaVoie, E. J.; Hoffmann, D. In *Polycyclic Hydrocarbons and Carcinogenesis*, ACS Monograph No. 283; Harvey, R. G., Ed.; American Chemical Society: Washington, DC, 1985; p 85.
(2) DiGiovanni, J.; Diamond, L.; Harvey, R. G.; Slaga, T. J. *Carcinogenesis* **1983**, *4*, 403. Sawyer, T. W.; Chang, K.; Harvey, R. G.; DiGiovanni, J. *Cancer Lett.* **1987**, *36*, 317.
(3) Syntheses of 1a and 1b were reported earlier: Harvey, R. G.; Cortez, C.; Jacobs, S. A. *J. Org. Chem.* **1982**, *47*, 2120. Konieczny, M.; Harvey, R. G. *J. Org. Chem.* **1980**, *45*, 1308.

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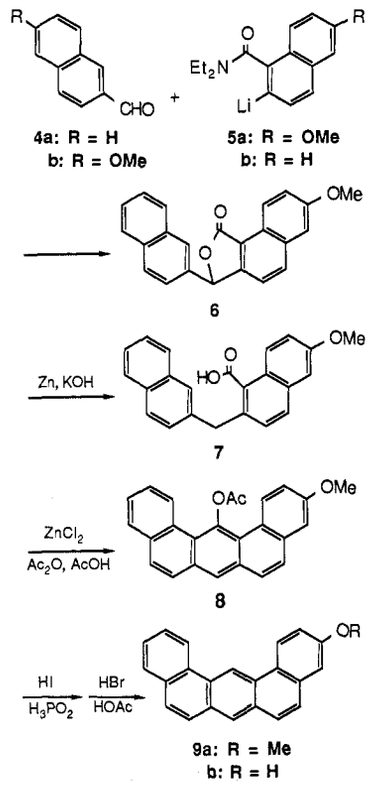
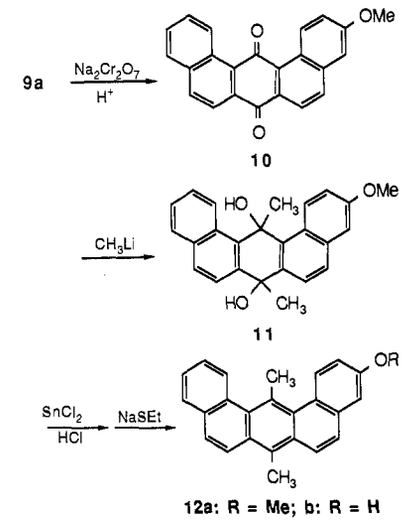
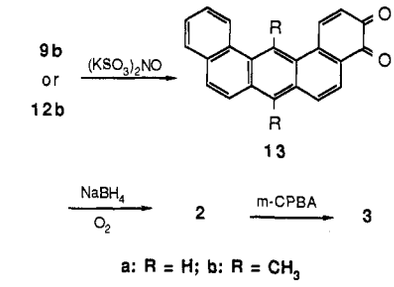
**Scheme I**




**Scheme II**




**Scheme III**




2-naphthaldehyde (**4a**) in ether at −78 °C gave the addition product, which on acidic treatment yielded the lactone **6**. Reduction of **6** with zinc and alkali furnished the free acid **7**. Cyclization of **7** with $ZnCl_2$ in acetic acid–acetic anhydride gave 3-methoxy-14-acetoxydibenz[*a,j*]anthracene (**8**). Deacetoxylation of **8** was accomplished smoothly and quantitatively by treatment with hydriodic acid in the presence of hypophosphorus acid in refluxing acetic acid to yield 3-methoxydibenz[*a,j*]anthracene (**9a**). A short reaction time (1.5 min) was employed to limit further reduction of the central meso ring.[8] Demethylation of **9a** with HBr in acetic acid yielded the free phenol **9b**.

Yields in excess of 90% were obtained in all steps except in the initial reaction of **5a** with 2-naphthaldehyde, which gave **6** in 60% yield. However, the alternative synthesis of **6** via condensation of *N,N*-diethyl-2-lithio-1-naphthamide (**5b**) with 6-methoxy-2-naphthaldehyde (**4b**) was less satisfactory, affording the corresponding lactone in only 28% yield.

7,14-Dimethyl-3-hydroxydibenz[*a,j*]anthracene (**12b**) required as the starting compound for the synthesis of **2b** was itself synthesized from **9a** via the synthetic approach in Scheme II. Oxidation of **9a** with chromic acid furnished the corresponding quinone, 3-methoxydibenz[*a,j*]-anthracene-7,14-dione (**10**). Addition of methyllithium to **10** followed by reduction of the adduct (**11**) with $SnCl_2$ and HCl[9] yielded 7,14-dimethyl-3-methoxydibenz[*a,j*]-anthracene (**12a**). Demethylation of **12a** with sodium ethylthiolate provided the free phenol **12b** in good overall yield.

Conversion of the phenols to the corresponding *trans*-dihydrodiols was accomplished by the two step sequence in Scheme III. Oxidation of **9b** with Fremy's salt [$(SO_3K)_2NO$] in a two phase aqueous methylene chloride system[5,6] furnished dibenz[*a,j*]anthracene-3,4-dione (**13a**). Reduction of this quinone with $NaBH_4$ in ethanol in the presence of $O_2$ took place smoothly and stereospecifically to provide the *trans*-dihydrodiol **2a**. This reaction was conducted in subdued light to minimize potential photooxidation.[10] The use of $O_2$ for the reoxidation of any catechol byproducts back to quinones has been previously demonstrated.[6,11] Similar oxidation of **12b** with Fremy's salt gave 7,14-dimethyldibenz[*a,j*]anthracene-3,4-dione (**13b**), which underwent reduction with $NaBH_4/O_2$ to yield the related *trans*-dihydrodiol **2b**.

The *trans*-dihydrodiol **2a** was converted stereospecifically to its bay region *anti*-diol epoxide **3a** by treatment with *m*-chloroperbenzoic acid. Attempted synthesis of the analogous dimethyl-substituted *anti*-diol epoxide **3b** by treatment of **2b** with *m*-chloroperbenzoic acid failed to afford **3b**, even with the mild conditions for isolation earlier utilized successfully for the preparation of the structurally related reactive bay region *anti*-diol epoxide derivative of 7,12-dimethylbenz[*a*]anthracene.[10] Apparently **3b** is unstable due to the strong steric interaction of the methyl group with both the angular aromatic ring and the saturated diol epoxide ring, which leads to substantial distortion from planarity. The analogous bay region *anti*-diol epoxide of 7,12-dimethylbenz[*a*]-anthracene, which is less sterically strained and lacks the angular aromatic ring of **3b**, is itself relatively unstable and decomposes rapidly following its isolation.[10]

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(7) Beak, P.; Brown, R. A. *J. Org. Chem.* **1977**, *42*, 1823; **1979**, *44*, 4463.
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Table I. Tumor-Initiating Activities of Dibenz[a,j]anthracene Derivatives[a]

| compound | dose, nmol | papillomas per mouse | % of mice with papillomas |
|---|---|---|---|
| THF | 0.2 mL | 0.05 | 5 |
| **1a** | 400 | 0.58[b] | 29 |
| **1b** | 400 | 17.3 | 97 |
| | 100 | 8.4 | 97 |
| **2a** | 400 | 0.65[b] | 39 |
| **2b** | 400 | 14.7 | 100 |
| | 100 | 9.9 | 93 |
| **3a** | 400 | 3.95 | 92 |

[a] All compounds were dissolved in peroxide-free THF (except **1b**, which was dissolved in acetone[2]). Twenty-four mice were used for each experimental group, except for the groups treated with **1b**, which contained 30 mice each.[2] Mice were initiated with the compound and dose indicated and 2 weeks later received a twice-weekly application of 3.4 nmol of 12-*O*-tetradecanoylphorbol-13-acetate (TPA). Data are given after 14 weeks of promotion, except for **1b**, which is given after 20 weeks of promotion.[2] Survival was ≥96% in all groups at 14 or 20 (compound **1b**) weeks of promotion. [b] Significantly different from THF only control group ($p < 0.05$) but not significantly different from each other ($p > 0.05$).

The 500-MHz $^1$H NMR spectra of the *trans*-dihydrodiols **2a** and **2b** and the *anti*-diol epoxide **3a** were entirely consistent with their structural assignments. The large value of the coupling between the carbinol protons of **2a** and **2b** ($J_{3,4}$ = 10.8 and 11.0 Hz, respectively) indicates that these dihydro diols exist predominantly in the diequatorial conformation.[12] This is consistent with previous findings for other sterically unhindered dihydrodiol derivatives of polycyclic hydrocarbons.[12,13] The lower value of the related couplings of the diol epoxide derivative **3a** ($J_{3,4}$ = 7.9 Hz) indicate a shift of the conformational equilibrium closer to a more nearly equal ratio of the diaxial and diequatorial conformers in the solutions of these compounds.

**Biological Activity.** The skin tumor initiating activities of the synthetic dihydrodiols **2a** and **2b** and the bay region *anti*-diol epoxide derivative **3a** were determined in female SENCAR mice. The data are shown in Table I along with similar data on the parent hydrocarbons. It should be noted that compound **1b** was not run concurrently with the other compounds in Table I. These data are taken from a previous report from other laboratories.[2] The *trans*-3,4-dihydrodiol **2a** exhibited tumorigenic activity approximately equal to that of the parent hydrocarbons **1a**. An initiating dose of 400 nmol of **2a** produced a maximal response of 0.65 papilloma per mouse after 14 weeks of promotion. Dibenz[a,j]anthracene (**1a**) at the same dose produced 0.58 papilloma per mouse. In contrast, the dimethyl-substituted dihydrodiol, **2b** induced 14.7 and 9.9 papillomas per mouse at doses of 400 and 100 nmol, respectively. This level of activity equalled approximately that of the parent hydrocarbon **1b** (17.3 and 8.4 papillomas per mouse at 400 and 100 nmol doses, respectively), although these two compounds were not compared directly in the same experiment. Nevertheless, the tumor-initiating activity of **2b**, at the 400 nmol per mouse dose, fell within the range of activity for the parent compound as determined in our previous studies.[2] The diol epoxide derivative of dibenz[a,j]anthracene **3a** exhibited significantly higher tumorigenicity than its dihydrodiol precursor **2a**. An initiating dose of 400 nmol of **3a** produced a maximal response of 3.95 papillomas per mouse. These findings are consistent with the hypothesis that bay region diol epoxide metabolites are the active carcinogenic forms of these hydrocarbons. The greater tumorigenicity of 7,14-dimethyldibenz[a,j]anthracene and its dihydrodiol derivative **2b** in comparison with their unsubstituted analogue **1a** and **2a** provides further support for the generalization that methyl substitution in bay regions enhances carcinogenic activity.[1,2]

## Experimental Section

**Materials and Methods.** *N,N*-Diethyl-2-lithio-6-methoxy-1-naphthamide (**5a**) was synthesized by metalation of the naphthamide derivative with *sec*-butyllithium by the method described.[14] Commercial *m*-chloroperbenzoic acid (Aldrich) was purified by washing with pH 7.5 phosphate buffer and drying under reduced pressure. Fremy's salt [$(KSO_3)_2NO$] was freshly prepared according to the published procedure.[15] *N,N,N',N'*-tetramethylethylenediamine (TMEDA) was dried over $LiAlH_4$ and redistilled. Tetrahydrofuran (THF) was freshly distilled from benzophenone ketyl. Ether was dried over sodium.

The NMR spectra were obtained on the University of Chicago 500-MHz NMR spectrometer in $CDCl_3$ unless stated otherwise with tetramethylsilane as internal standard. Integration was consistent with all structural assignments. The ultraviolet spectra were measured in absolute ethanol on a Perkin-Elmer Lambda 5 spectrometer. Melting points are uncorrected. All new compounds gave satisfactory microanalyses for C, H within ±.03% and/or mass spectra consistent with the assigned structures.

**Synthesis of the Lactone 6.** A solution of **5a** in diethyl ether (150 mL) was prepared by metalation of *N,N*-diethyl-6-methoxy-1-naphthamide (3.37 g, 13.2 mmol) with *sec*-butyllithium and TMEDA (2.3 mL) by the method described.[14] To this solution at −78 °C under argon was added a solution of 2-naphthaldehyde (3.09 g, 19.9 mmol) in anhydrous THF (50 mL), and the resulting solution was stirred at this temperature for 3 h and at ambient temperature overnight. The usual workup gave the crude product, which was dissolved in dry benzene along with *p*-toluenesulfonic acid (10% by wt). The solution was heated at reflux for 5 h and then passed through a column of silica gel to yield the lactone **6** (2.7 g, 60%): mp 195.5–196.5 °C; NMR δ 3.94 (s, 3, $CH_3$), 6.57 (s, 1, methine), 7.24–7.96 (m, 11, aromatic), 8.98 (d, 1, H, $J_{1,2}$ = 9.1 Hz). Anal. Calcd for $C_{23}H_{16}O_3$: C, 81.16; H, 4.74. Found: C, 80.96; H, 4.78.

**Reduction of 6 to the Carboxylic Acid 7.** A solution of **6** (2.5 g, 7.3 mmol) in pyridine (20 mL) was added to a mixture of preactivated zinc dust (25 g) in a solution of 10% KOH (200 mL). The mixture was stirred at reflux overnight, cooled, and filtered. The filtrate was worked up in the usual manner to afford the free acid **7** (2.3 g, 92%): mp 138–140 °C; NMR δ 3.82 (s, 3, $CH_3$), 4.29 (s, 2, $CH_2$), 6.9–8.2 (m, 12, aromatic), 8.61 (br s, 1, OH). Anal. Calcd for $C_{23}H_{18}O_3$: C, 80.68; H, 5.30. Found: C, 80.75; H, 5.36.

**3-Methoxy-14-acetoxydibenz[a,j]anthracene (8).** To a solution of **7** (2.0 g, 5.8 mmol) in acetic anhydride (15 mL) and glacial acetic acid (30 mL) was added $ZnCl_2$ (300 mg), and the mixture was heated at reflux for 1 h. The product was precipitated by addition of ice water, dried, and recrystallized from benzene–hexane to yield **8** (2.0 g, 93%): mp 195–196 °C; NMR δ 2.52 (s, 3, $CH_3CO$), 3.96 (s, 3, $CH_3O$), 7.2–8.2 (m, 12, aromatic), 9.30 (d, 1, $H_{1 \text{ or } 13}$, $J$ = 10.1 Hz), 9.37 (d, 1, $H_{1 \text{ or } 13}$, $J$ = 8.5 Hz). Anal. Calcd for $C_{25}H_{18}O_3$: C, 81.95; H, 4.95. Found: C, 81.77; H, 5.01.

**3-Methoxydibenz[a,j]anthracene (9a).** A solution of 57% HI (5.5 g) and 50% hypophosphorous acid (3 g) and acetic acid (100 mL) was brought to reflux and added to a suspension of **8** (1.7 g, 4.6 mmol) in acetic acid (100 mL) at 100 °C. The reaction mixture was stirred at this temperature for 90 s, whereupon it decolorized. The clear solution was poured into ice water and worked up coventionally to yield **9a** (1.4 g, 100%): mp 169.5–170.5

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(14) Jacobs, S. A.; Cortez, C.; Harvey, R. G. *Carcinogenesis* **1983**, *4*, 519.
(15) Zimmer, H.; Lankin, D. C.; Horgan, S. W. *Chem. Rev.* **1971**, *71*, 229.

°C (benzene–hexane); NMR δ 3.98 (s, 3, $CH_3$), 7.2–8.0 (m, 9, aromatic), 8.29 (s, 1, $H_7$), 8.86 (d, 1, $H_{1\text{ or }13}$, $J$ = 8.8 Hz), 8.94 (d, 1, $H_{1\text{ or }13}$, $J$ = 8.0 Hz), 9.87 (s, 1, $H_{14}$). Anal. Calcd for $C_{23}H_{16}O$: C, 89.58; H, 5.23. Found: C, 89.40; H, 5.23.

**3-Hydroxydibenz[*a*,*j*]anthracene (9b).** To a solution of **9a** (1.4 g, 4.5 mmol) in refluxing acetic acid (180 mL) was added HBr (60 mL) dropwise over 20 min. The mixture was heated at reflux for 3.5 h, cooled, and then poured into ice water. The precipitate was worked up conventionally to afford **9b** (1.32 g, 100%). A portion was dissolved in $CH_2Cl_2$, poured through a Florisil column, and recrystallized from benzene–hexane to yield pure **9b**: mp 237–238 °C; NMR (acetone-$d_6$ + $D_2O$) δ 7.3–8.0 (m, 9, aromatic), 8.42 (s, 1, $H_7$), 9.1 (d, 1, $H_{1\text{ or }13}$), 9.2 (d, 1, $H_{1\text{ or }13}$), 10.09 (s, 1, $H_{14}$). Anal. Calcd for $C_{22}H_{14}O$: C, 89.77; H, 4.79. Found: C, 89.62; H, 4.84.

**3-Methoxydibenz[*a*,*j*]anthracene-7,14-dione (10).** A solution of **8** (2.1 g, 5.7 mmol) and $Na_2Cr_2O_7$ (3.0 g) in glacial acetic acid was heated at reflux for 15 min. The reaction mixture was cooled, and then dilute 30% sulfuric acid (90 mL) was added. The yellow precipitate was filtered, washed with water, and dried. The crude product was dissolved in $CHCl_3$–benzene, passed through a column of Florisil, and eluted with $CHCl_3$ to yield **10** (1.7 g, 90%): mp 193–194 °C (acetone–$CH_3OH$); NMR ($Me_2SO$-$d_6$) δ 3.95 (s, 3, $CH_3$), 7.3–8.4 (m, 9, aromatic), 9.06 (d, 1, $H_{1\text{ or }13}$), 9.16 (d, 1, $H_{1\text{ or }13}$). Anal. Calcd for $C_{23}H_{14}O_3$: C, 81.64; H, 4.17. Found: C, 81.75; H, 4.17.

**7,14-Dimethyl-3-methoxydibenz[*a*,*j*]anthracene (12a).** To a solution of the quinone **10** (1.8 g, 5.3 mmol) in benzene (300 mL) was added methyllithium (38 mL of a 1.4 M solution in ether) under argon. The solution was stirred at room temperature overnight, neutralized with dilute HCl, and worked up in the usual manner.

To a solution of $SnCl_2$ (10 g) and concentrated HCl (6 mL) in ether (150 mL) was added the product of the foregoing reaction (1.96 g). The solution was stirred for 10 min, quenched with water, and worked up conventionally. A solution of the product in benzene was passed through a Florisil column to yield **12a** (0.89 g, 50%): mp 161.5–162.5 °C; NMR δ 3.10 (s, 3, 7-$CH_3$), 3.58 (s, 3, 14-$CH_3$), 3.99 (s, 3, $CH_3O$), 7.2–8.2 (m, 9, aromatic), 8.84 (d, 1, $H_{1\text{ or }13}$), 8.89 (d, 1, $H_{1\text{ or }13}$). Anal. Calcd for $C_{25}H_{20}O$: C, 89.24; H, 5.99. Found: C, 89.34; H, C, 6.17.

**3-Hydroxy-7,14-dimethyldibenz[*a*,*j*]anthracene (12b).** A solution of ethanethiol (552 mg, 8.9 mmol) in diethylformamide (5 mL) was added dropwise from an addition funnel to a solution of NaH (214 mg, 8.9 mmol) in DMF (5 mL) under $N_2$. The solution was stirred for 5 min, and then a solution of **12a** (300 mg, 0.89 mmol) in DMF (15 mL) was added. The solution was heated at reflux for 3 h, cooled, diluted with water, acidified with HCl, and worked up in the usual manner. The crude product was triturated with 3 portions of hexane, dissolved in benzene, and chromatographed on a Florisil column to yield **12b** (230 mg, 80%): mp 208–209 °C; NMR (acetone-$d_6$) δ 3.09 (s, 3, 7-$CH_3$), 3.57 (s, 3,14-$CH_3$), 7.2–8.2 (m, 9, aromatic), 8.88 (d, 1, $H_{1\text{ or }13}$), 8.98 (d, 1, $H_{1\text{ or }13}$). Anal. Calcd for $C_{24}H_{18}O$: C, 89.41; H, 5.63. Found: C, 89.25; H, 5.66.

**Dibenz[*a*,*j*]anthracene-3,4-dione (13a).** A solution of Fremy's salt (1.5 g, 5.6 mmol) in $^1/_6$ M $KH_2PO_4$ (90 mL) was added to a stirred solution of **9b** (500 mg, 1.7 mmol) and Adogen 464 (0.5 mL) in $CH_2Cl_2$ (10 mL) and benzene (40 mL) under argon. The deep purple solution was stirred for 1 h. The product was extracted into $CH_2Cl_2$, washed with water, and evaporated to dryness. Trituration with acetone gave **13a** (400 mg, 80%): mp >260 °C; NMR ($Me_2SO$-$d_6$) δ 6.62 (d, 1, vinylic), 7.6–8.5 (m, 9, aromatic + vinylic), 8.63 (s, 1, $H_7$), 9.94 (s, 1, $H_{14}$). Anal. Calcd for $C_{22}H_{12}O_2$: C, 85.70; H, 3.92. Found: C, 85.57; H, 3.98.

***trans*-3,4-Dihydroxy-3,4-dihydrodibenz[*a*,*j*]anthracene (2a).** $NaBH_4$ (10 g) was added to a stirred suspension of the quinone **13a** (0.4 g, 1.3 mmol) in ethanol (500 mL). The mixture was stirred with a stream of $O_2$ bubbling through at room temperature for 40 h. The ethanol was evaporated on a rotary evaporator with a dry ice condenser. The product was extracted into ether–THF and acetylated with acetic anhydride (18 mL) and pyridine (2 mL) overnight. Addition of water precipitated the crude product (0.46 g), which was purified by chromatography on Florisil to yield **2a** diacetate (310 mg, 60%): mp 213–214 °C (benzene–hexane); NMR δ 2.06 (s, 3, $CH_3CO$), 2.14 (s, 3, $CH_3CO$), 5.69 (t, 1, $H_3$, $J_{3,4}$ = 4.9 Hz), 6.34 (d of d, 1, $H_2$, $J_{1,2}$ = 10.0 Hz, $J_{2,3}$ = 4.2 Hz), 6.36 (d, 1, $H_4$), 7.4–7.9 (m, 10, aromatic + $H_1$), 8.32 (s, 1, $H_7$), 8.82 (d, 1, $H_{13}$), 9.47 (s, 1, $H_{14}$); UV 235 nm (ε 41 470), 244 (43 700), 280 (51 670), 290 (71 750), 302 (61 740), 368 (7130). Anal. Calcd for $C_{26}H_{20}O_4$: C, 78.78; H, 5.08. Found: C, 78.65; H, 5.14.

The diacetate was dissolved in THF (20 mL), and to this solution was added a suspension of $NaOCH_3$ (200 mg, 3.6 mmol) in $CH_3OH$ (100 mL). The reaction mixture was heated at reflux for 10 min, worked up in the usual manner, avoiding heating, and triturated with ether to yield pure **2a** (160 mg, 86%): mp 224–226 °C; NMR ($Me_2SO$-$d_6$) δ 4.41 (d, 1, $H_3$, $J_{3,4}$ = 10.8 Hz), 4.79 (d, 1, $H_4$), 6.29 (d of d, 1, $H_2$, $J_{1,2}$ = 10.8 Hz, $J_{2,3}$ = 2.5 Hz), 7.6–8.1 (m, 8, aromatic + $H_1$), 8.48 (s, 1, $H_7$), 9.16 (d, 1, $H_{13}$), 9.58 (s, 1, $H_{14}$); UV 236 nm (ε 42 170), 245 (55 350), 279 (48 200), 289 (71 700), 301 (66 500), 370 (7500). Anal. Calcd for $C_{22}H_{16}O_2$: C, 84.59; H, 5.16. Found: C, 84.35; H, 5.22.

***trans*-3,4-Dihydroxy-*anti*-1,2-epoxy-1,2,3,4-tetrahydrodibenz[*a*,*j*]anthracene (3a).** A solution of **2a** (103 mg, 0.33 mmol) in THF (100 mL) was treated with *m*-chloroperbenzoic acid (567 mg, 3.3 mmol) and stirred at ambient temperature under $N_2$ for 1 h. The reaction was quenched by addition of cold water and worked up rapidly to minimize hydrolysis of the diol epoxide. The product was extracted into ether, washed three times with cold 10% NaOH, two times with cold water, dried, and evaporated, avoiding heating. The product was triturated with ether to yield **3a** (65 mg, 60%): mp 204.5–205.5 °C dec; NMR ($Me_2SO$-$d_6$ + $D_2O$) δ 3.82 (d, 1, $H_2$, $J_{1,2}$ = 4.4 Hz), 3.90 (d, 1, $H_3$, $J_{3,4}$ = 7.9 Hz), 4.54 (br d, 1, $H_4$), 5.52 (br d, 1, $H_1$), 7.6–8.2 (m, 7, aromatic), 8.54 (s, 1, $H_7$), 9.26 (d, 1, $H_{13}$), 9.84 (s, 1, $H_{14}$); UV 225 nm (ε 42 380), 234 (37 050), 262 (37 700), 271 (45 180), 281 (82 170), 293 (104 000), 352 (7200). Anal. Calcd for $C_{22}H_{16}O_3$: C, 80.47; H, 4.91. Found: C, 80.27; H, 4.93.

**7,14-Dimethyldibenz[*a*,*j*]anthracene-3,4-dione (13b).** Oxidation of **12b** (214 mg, 0.66 mmol) with Fremy's salt (1.0 g, 4 mmol) by the procedure employed for the oxidation of **9b** furnished **13b** (200 mg, 89%): mp 208–209 °C; NMR δ 3.04 (s, 3, 7-$CH_3$), 3.3 (s, 3, 14-$CH_3$), 6.44 (d, 1, $H_2$), 7.6–8.7 (m, 9, aromatic + $H_1$). Anal. Calcd for $C_{24}H_{16}O_2$: C, 85.70; H, 4.80. Found: C, 84.56; H, 4.85.

***trans*-3,4-Dihydroxy-3,4-dihydro-7,14-dimethyldibenz[*a*,*j*]anthracene (2b).** Reduction of **13b** (260 mg, 0.77 mmol) with $NaBH_4$ (570 mg) by the method described for the reduction of **13a** gave **2b** (180 mg, 69%): mp 224.5–225.5 °C; NMR ($Me_2SO$-$d_6$) δ 3.01 (s, 3, 7-$CH_3$), 3.22 (s, 3, 14-$CH_3$), 4.44 (d, 1, $H_3$, $J_{3,4}$ = 11.0 Hz), 4.60 (d of d, 1, $H_4$), 5.23 (br d, 1, OH), 5.60 (br d, 1, OH) 6.10 (d of d, $H_2$, $J_{1,2}$ = 10.1 Hz, $J_{2,3}$ = 2.1 Hz), 7.31 (d of d, 1, H), the peaks at 5.23 and 5.60 disappeared in the presence of $D_2O$; UV 221 nm (ε 32 960), 253 (34 640), 301 (59 980), 315 (58 300), 394 (10 090). Anal. Calcd for $C_{24}H_{20}O_2$: C, 84.68; H, 5.92. Found: C, 84.51; H, 5.91.

A portion of the above dihydrodiol was acetylated with acetic anhydride and pyridine to provide **2b** diacetate: mp 207–208 °C; NMR δ 2.09 (s, 3, $CH_3CO$), 2.17 (s, 3, $CH_3CO$), 3.04 (s, 3, 7-$CH_3$), 3.30 (s, 3, 14-$CH_3$), 5.72 (m, 1, $H_3$), 6.15 (d of d, 1, $H_2$, $J_{1,2}$ = 10.1 Hz, $J_{2,3}$ = 3.8 Hz), 6.36 (d, 1, $H_4$, $J_{3,4}$ = 7.0 Hz), 7.4–8.3 (m, 9, aromatic + $H_1$), 8.70 (m, 1, $H_{14}$); UV 222 nm (ε 30 680), 230 (30 400, 253 (31 100), 301 (60 880), 314 (70 660), 395 (6450). Anal. Calcd for $C_{28}H_{24}O_4$: C, 79.22; H, 5.70. Found: C, 79.07; H, 5.74.

**6-Methoxy-2-naphthaldehyde (4b).** To a solution of 2-bromo-6-methoxynaphthalene (Aldrich) (11 g, 46 mmol) in dry ether (500 mL) at −78 °C under an argon atmosphere was added *n*-butyllithium (22 mL of a 2.5 M solution in hexane, 55 mmol). After 5 min the bath was removed, and stirring was continued for 1 h. The solution was again cooled to −78 °C, and dimethylformamide (20 mL) was added. After 5 min the bath was removed, and stirring was continued for 2 h; the reaction was worked up as usual to furnish **4b** (8.5 g, 100%): mp 82–83 °C (benzene–hexane); NMR δ 3.96 (s, 3, $CH_3$), 7.1–9.5 (m, 6, aryl), 10.13 (s, 1, CHO). Anal. Calcd for $C_{12}H_{10}O_2$: C, 77.40; H, 5.41. Found: C, 77.23; H, 5.42.

**Tumor-Induction Experiments.** Female SENCAR mice (purchased from Research Biogenics, Inc., Bastrop, TX) were used for all experimental groups. At 7–9 weeks of age, the backs of the mice were shaved with surgical clippers. Mice were allowed to rest for 2 days, and only those mice in the resting phase of the

hair growth cycle were used. All initiators were applied topically to the shaved region in 0.2 mL of peroxide-free THF, and control mice received solvent only. Mice were initiated with various doses of each test compound followed 2 weeks later by twice-weekly applications of 3.4 nmol of TPA in 0.2 mL of acetone. Development of skin papillomas was observed and recorded weekly. Papillomas were removed at random for histological verification. The data in this study are presented as the average number of papillomas per mouse and the percent of mice with papillomas. Statistical analyses of differences between mean papilloma responses were performed (where appropriate) with the Student's $t$ test. The level of significance was set at $p \leq 0.05$.

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**Registry No.** **2a**, 114326-33-9; **2a** diacetate, 114326-32-8; **2b**, 114326-36-2; **2b** diacetate, 114350-59-3; **3a**, 114326-34-0; **4a**, 66-99-9; **4b**, 3453-33-6; **6**, 114326-26-0; **7**, 114326-27-1; **8**, 114326-28-2; **9a**, 83136-28-1; **9b**, 83136-32-7; **10**, 76214-37-4; **12a**, 114326-29-3; **12b**, 114326-30-6; **13a**, 114326-31-7; **13b**, 114326-35-1; *N,N*-diethyl-6-methoxy-1-naphthamide, 114326-25-9; 2-bromo-6-methoxynaphthalene, 5111-65-9.

# Heterocyclic Muscarinic Agonists. Synthesis and Biological Activity of Some Bicyclic Sulfonium Arecoline Bioisosteres

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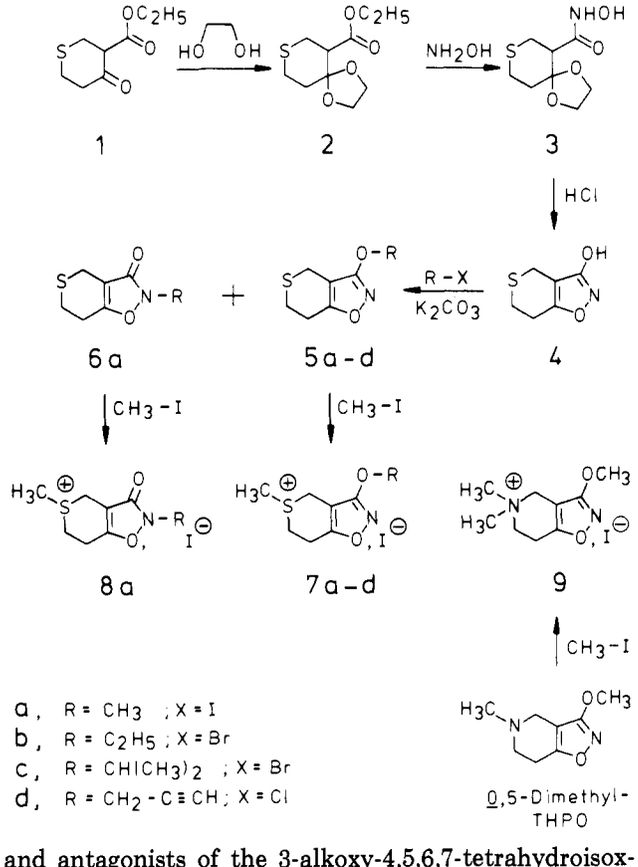
A number of *S*-methylsulfonium analogues of the conformationally restricted muscarinic agonists of the 3-alkoxy-4,5,6,7-tetrahydroisoxazolo[4,5-*c*]pyridine (*O*-alkyl-THPO) type have been synthesized. The effects on muscarinic receptors of these 3-alkoxy-5-methyl-6,7-dihydro-4*H*-thiopyrano[3,4-*d*]isoxazol-5-ium (*O*-alkyl-*S*-methyl-DHTO) analogues (**7a–d**) were assessed in receptor-binding experiments with tritiated oxotremorine M, pirenzepine, and quinuclidinyl benzilate as ligands and were supported by studies on the isolated guinea pig ileum. The degree of muscarinic agonist activity of the compounds (M-agonist index) and their selectivity for M-1 or M-2 muscarinic receptor subtypes (M-2/M-1 index) were estimated on the basis of receptor-binding studies. The in vitro pharmacological profiles of the compounds were compared with those of arecoline and its sulfonium and 3-methoxyisoxazole isosteres, sulfoarecoline and *O*,5-dimethyl-THPO, respectively. While *O*-methyl-DHTO (**5a**) and *N*-methyl-DHTO (**6a**) were inactive, all of the sulfonium analogues **7a–d** were muscarinic agonists with the exception of *O*-ethyl-*S*-methyl-DHTO (**7b**), which showed a muscarinic antagonist profile.

There is strong evidence of major deficits in central cholinergic transmission in patients with the pathology characteristic of Alzheimer's disease (AD) and senile dementia of the Alzheimer type (SDAT).[1-5] On the basis of clinical and animal behavioral studies, this cholinergic deficit may be of particular relevance to disturbances in learning and memory in AD/SDAT patients.[1,3,4,6] Neurochemical examination of autopsy and biopsy brain material from Alzheimer patients have revealed loss of the presynaptic marker enzyme, choline acetyltransferase, and presynaptic muscarinic receptor sites of the M-2 subtype correlating with dementia score and severity of neurohistopathology. Postsynaptic muscarinic receptor sites, primarily of the M-1 subtype, do, however, seem to survive the loss of cholinergic nerve terminals.[1,2,7] There may actually be an up-regulation of postsynaptic M-1 receptors in Alzheimer patients.[8] Although the degree of functional integrity of muscarinic receptors in AD/SDAT brains is, as yet, unknown, much interest is focused on such receptors as therapeutic sites of attack. Whereas antagonists at presynaptic M-2 receptors might be useful drugs at the early stages of AD/SDAT,[9] agonists at postsynaptic M-1 receptors or, perhaps, compounds with mixed M-1 agonist/M-2 antagonist profiles appear to be of particular therapeutic interest.[8,10]

These aspects have accelerated the pharmacological characterization of M-1 and M-2 receptors.[11] As part of our attempts to elucidate the muscarinic pharmacophore(s) relevant to AD/SDAT, we have developed a series of potent and conformationally restricted muscarinic agonists and antagonists of the 3-alkoxy-4,5,6,7-tetrahydroisoxazolo[4,5-*c*]pyridine (*O*-alkyl-THPO) type.[12-15]

**Scheme I**

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