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SYNTHESIS OF BIOLOGICALLY ACTIVE DIHYDRODIOL METABOLITES OF 16,17-DIHYDRO-15*H*-CYCLOPENTA[a]PHENANTHRENE AND ITS CARCINOGENIC 11-METHYL AND 17-KETO DERIVATIVES

Robert J. Young, Cecilia Cortez, Ernestina Luna, H. Lee, and Ronald G. Harvey*
Ben May Institute, University of Chicago, Chicago, Illinois 60637

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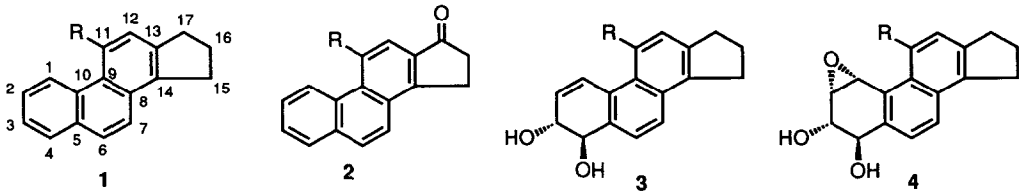
Abstract: Syntheses are described of the *trans*-3,4-dihydrodiol metabolites of the title compounds implicated by biological studies as proximate carcinogenic forms that give rise to the corresponding diol epoxide metabolites that bind covalently to DNA *in vivo*.

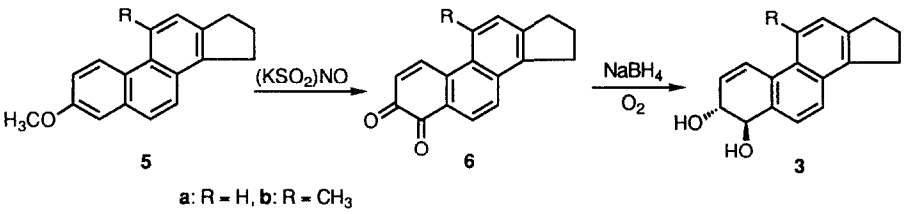
Cyclopenta[a]phenanthrenes are widely distributed in petroleum and other natural environments where they are thought to arise from sterols by microbiological transformation.[1,2] There is evidence that compounds of this class enter into the human diet by pyrolysis of sterols in edible oils during cooking.[2,3] While the parent hydrocarbon (**1a**) is inactive as a carcinogen, its 11-methyl-17-keto derivative (**2b**)[4] is a relatively potent carcinogen on mouse skin, comparable in activity to benzo[a]pyrene.[5] The mechanism of carcinogenesis is thought to involve activation by the microsomal P-450 enzymes to dihydrodiol metabolites, e.g. **3**, that undergo further metabolic transformation to mutagenic diol epoxides, e.g. **4**, that bind covalently to DNA and are the ultimate carcinogenic forms.[2,6] However, biological studies have been severely hampered by the synthetic inaccessibility of the dihydrodiol and diol epoxide metabolites.



1 **2** **3** **4**

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

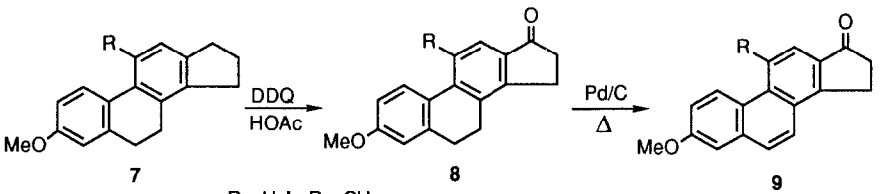
We now report the first successful synthesis of the *trans*-3,4-dihydrodiol derivatives of the cyclopenta[a]phenanthrene ring system, including that of the highly carcinogenic 16,17-dihydro-11-methyl-17-oxo-15*H*-cyclopenta[a]phenanthrene (**2b**). The synthetic strategy entailed initial preparation of the 3-methoxy derivatives of **1a** and **1b** from 6-methoxy-1-tetralone via appropriate modification of the route reported earlier for the preparation of the parent hydrocarbons.[7] Conversion of these key intermediates (**5a** and **5b**) to the corresponding *trans*-3,4-dihydrodiol derivatives, **3a** and **3b**, was accomplished by demethylation with NaSEt in DMF followed by oxidation of the resulting phenols to the corresponding o-quinones, **6a** and **6b**, with Fremy's salt,[8,9] and stereospecific *trans*-reduction with $NaBH_4/O_2$.[8,10]



5 **6** **3**

a: R = H, **b**: R = CH_3

The synthetic route to the corresponding *trans*-3,4-dihydrodiol derivatives of 16,17-dihydro-15*H*-17-oxo-cyclopenta[a]phenanthrene and its 11-methyl derivative required the 17-keto analogs of **5a** and **5b** (**9a** and **9b**) as starting compounds. It was previously found that while direct oxidation of **1a** with DDQ in moist acetic acid[11] afforded a mixture of the 15- and 17-keto derivatives, oxidative attack regiospecifically in the 17-position could be effected by prior hydrogenation of the 6,7-bond. Hydrogenation of **5a** over 10% Pd/C (a K-region specific catalyst)[12] afforded the 6,7-dihydro derivative (**7a**). Oxidation of **7a** with DDQ in moist acetic acid gave the corresponding 17-keto derivative (**8a**) which underwent dehydrogenation over the same catalyst to yield 16,17-dihydro-15*H*-17-oxo-cyclopenta[a]phenanthrene (**9a**) in good overall yield.[11] A similar synthetic sequence was employed to prepare the 11-methyl analog (**9b**) from **5b**. The assignment of **9b** as the 17-keto isomer is supported by its 500 MHz proton NMR spectrum in which the bay region H_7 aromatic proton peak appeared at δ 7.83, shifted only slightly downfield from the signal of its precursor **5b** (δ 7.65), distinguishing it from the 15-keto isomer in which the H_7 peak is expected to be strongly deshielded by the adjacent carbonyl function.[13]



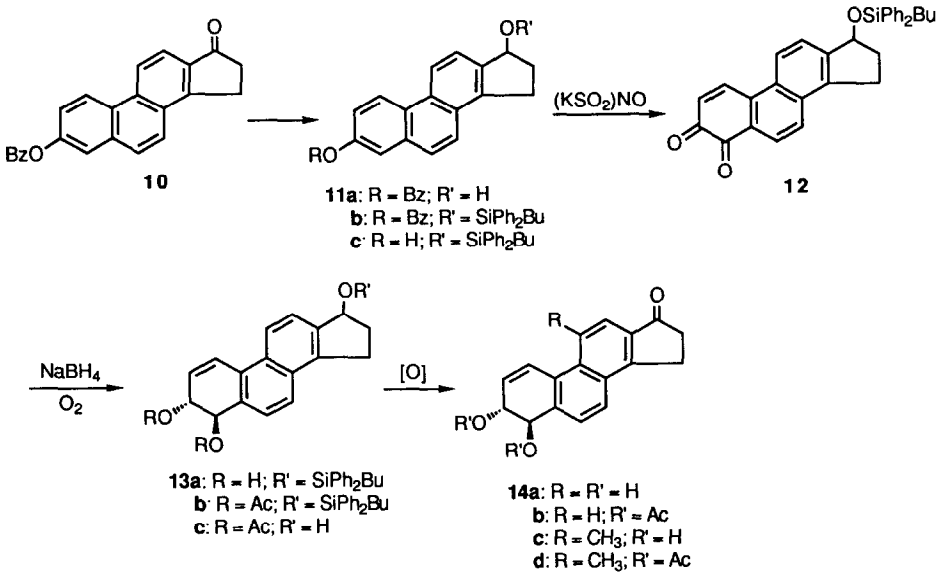
7 **8** **9**

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

Conversion of **9a** to the corresponding *trans*-3,4-dihydrodiol (**14a**) necessitated appropriate protection of the oxygen functions. Protection of the 3-hydroxyl group as a methyl ether was unsatisfactory because of the harshness of the reagents required for its later removal, and protection of the 17-keto function by a ketal gave an unstable product that proved unsuitable. The successful strategy involved demethylation of **9a** with BBr_3, formation of the benzoate ester **10**, reduction of the carbonyl function with $NaBH_4$ in THF-MeOH, and reaction of the alcohol product **11a** with *t*-butyldiphenylsilyl chloride and pyridine to afford the silylated derivative **11b**. Debenzoylation of **11b** by treatment with methanolic KOH gave the free phenol **11c** which in turn was oxidized with Fremy's salt to yield the quinone **12**. Reduction of **12** with $NaBH_4/O_2$ by the procedure employed for preparation of the dihydrodiols **6a** and **6b** provided the *trans*-3,4-dihydrodiol derivative of 17-(*t*-butyldiphenylsilyloxy)cyclopenta[a]phenanthrene (**13a**).

Transformation of **13a** to the *trans*-3,4-dihydrodiol derivative of 16,17-dihydro-15*H*-17-oxocyclopenta[a]phenanthrene (**14a**) required protection of the dihydrodiol function prior to oxidation of the protected 17-

hydroxyl group. This was accomplished by acetylation of the dihydrodiol function to give **13b** which underwent deprotection of the silyl group on treatment with tetrabutylammonium fluoride to provide **13c**. Oxidation of the 17-hydroxyl group of **13c** with the Dess-Martin periodinane reagent,[14] and deacetylation of the product with NaOMe afforded *trans*-3,4-dihydroxy-17-oxo-3,4,16,17-tetrahydro-15*H*-cyclopenta[a]phenanthrene (**14a**). Synthesis of the 11-methyl-substituted analogue of **14a** (**14c**) from **9b** was successfully accomplished via an analogous sequence of steps.[15] The structural assignments of all synthetic intermediates as well as the final products were fully consistent with their 500 MHz proton NMR spectra.[16]



The dihydrodiols synthesized herein have been utilized as starting compounds for the synthesis of the corresponding isomeric *anti*- and *syn*-diol epoxide derivatives implicated as the ultimate carcinogenic metabolites. These syntheses are currently being repeated on preparative scale. Preliminary findings from mutagenicity tests conducted by Dr. Costa Ioannides (University of Surrey, England) using the Ames assay with *Salmonella typimurium* TA100 bacteria with microsomal activation indicate that the dihydrodiols **3a** and **3b** are more mutagenic than the parent hydrocarbons, and the corresponding diol epoxides are highly mutagenic without activation. Similar assays will be conducted on **14a** and **14b** and the related diol epoxides in due course.

Acknowledgement. This research was supported by grants from the National Institute of Environmental Health Sciences (ES 04732) and the National Cancer Institute (CA 36097).

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10. The *trans*-stereospecificity of these reductions is well established.[8] The assignments of the dihydrodiols **3a** and **3b** were consistent with their 500 MHz proton NMR spectra (DMSO-d6): **3a** δ 2.20 (q, 2, H_{16}), 3.10 (t, 2, H_{17}), 3.25 (t, 2, H_{15}), 4.34 (m, 1, H_3), 4.71 (dd, 1, H_4), 5.23 (d, 1, OH), 5.56 (d, 1, OH), 6.12 (dd,1, H_2), 7.23 (d,1, H_{12}), 7.45 (dd,1, H_1), 7.73 (m, 2, $H_{6,7}$), 8.03 (d,1, H_{11}); $J_{1,2}$ = 8.7, $J_{2,3}$ = 2.0, $J_{3,4}$ = 8.4, $J_{11,12}$ = 9.1, $J_{15,16,17}$ = 7.3 Hz; UVmax (EtOH) 204 (17100), 237 (39800), 321 (2700), 348 (2500); mp 202-204°C; **3b** δ 2.12 (q, 2, H_{16}), 2.94 (t, 2, H_{17}), 3.10 (t, 2, H_{15}), 3.24 (s, 3, CH_3), 4.22 (m, 1, H_3), 4.47 (dd, 1, H_4), 5.08 (d, 1, OH), 5.43 (d, 1, OH), 5.96 (dd,1, H_2), 7.15 (s,1, H_{12}), 7.22 (dd,1, H_1), 7.61 (s, 1, H_{6or7}), 7.67(s,1, H_{6or7}); $J_{1,2}$ = 10.4, $J_{2,3}$ = 2.0, $J_{3,4}$ = 8.4, $J_{6,7}$ = 8.5, $J_{15,16,17}$ = 7.3 Hz; UVmax (EtOH) 204 (12300), 221 (10800), 244 (28200), 333 (4300); mp 188-190°C.
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12. Fu, P. P.; Lee, H. M.; Harvey, R. G. J. Org. Chem. **1980**, *45*, 2797.
13. The H7 bay region proton of the 17-keto derivative **2a** appeared at δ 7.83, whereas the related proton of its 15-keto analogue was found as a doublet at δ 9.16.[7]
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15. All new compounds were fully characterized. Good yields were obtained in all steps except for conversion of **13b** to **13c** which was 20-25%. It is anticipated that this can be improved with further study.
16. The NMR spectra (500 MHz) of the dihydrodiols and their diacetates were in agreement with their assignments: **14a** (DMSO-d_6) δ 2.76 (m, 2, H_{16}), 3.40 (m, 2, H_{15}), 4.36 (dd, 1, H_3), 4.70 (dd, 1, H_4), 5.28 (d, 1, OH), 5.72 (d, 1, OH), 6.18 (dd, 1, H_2), 7.25 (dd, 1, H_1), 7.60 (d, 1, H_{12}), 7.90 (d, 1, H_6), 8.08 (d, 1, H_7), 8.22 (d, 1, H_{11}); $J_{1,2}$ = 10.0, $J_{2,3}$ = 2.1, $J_{3,4}$ = 10.8, $J_{6,7}$ = 8.4; mp 310-12°C; **14b** ($CDCl_3$) δ 2.10 and 2.17 (pair s, 6, MeCO), 2.89 (m, 2, H_{16}), 3.47 (m, 2, H_{15}), 5.68 (dd, 1, H_3), 6.29 (dd, 1, H_2), 6.36 (d, 1, H_4), 7.45 (d, 1, H_1), 7.62 (d,1, H_6), 7.81 (d,1, H_{12}), 8.01 (d,1, H_7), 8.15 (d,1, H_{11}); $J_{1,2}$ = 10.1, $J_{2,3}$ = 4.0, $J_{3,4}$ = 6.2, $J_{6,7}$ = 8.4, $J_{11,12}$ = 8.9 Hz; mp 205-6°C; the NMR spectra of **14c** and **14d** were similar except for the 11-methyl group.