of 250 μA. All synthetic intermediates gave NMR and mass spectra consistent with the assigned structures.

(14) This entails the heterolytic fission of the cyclic endoperoxide (PGG_2 or PGH_2) resulting in an electron-deficient oxygen at C-9 which in turn attacks the 5(6) double bond with the formation of a 6,9α-oxy ring and a carbonium ion at C-5. Instead of losing a proton from C-6 to form PGI_2, a 1,2- or possibly a 1,3-hydride shift takes place to yield a carbonium ion at either C-6 or C-7, respectively, which upon loss of a proton from C-7 or C-6 affords **9**.

(15) Tetrahydro-PGI_2 methyl ester was prepared by hydrogenation of PGI_2 methyl ester over 10% Pd/C in ethanol containing 1% Et_3N for 1 h at 25 °C.

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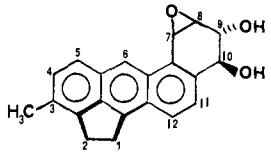
Metabolic Formation of 1,9,10-Trihydroxy-9,10-dihydro-3-methylcholanthrene: a Potential Proximate Carcinogen from 3-Methylcholanthrene

Sir:

3-Methylcholanthrene (MC), first synthesized by Fieser[1] in 1935, is one of the most potent carcinogenic polycyclic aromatic hydrocarbons.[2] The carcinogenicity of MC, like that of other polycyclic aromatic hydrocarbons, is believed to be due to its oxidative metabolism to one or more reactive species.[3-5] To date, several oxygenated metabolites of MC have been identified,[6] but the structure(s) of the chemically reactive, ultimate carcinogenic metabolite(s) of MC has yet to be determined. Previous studies from these laboratories have concentrated on the identification of the ultimate carcinogens from the substituted, alternant hydrocarbons. Data obtained with derivatives of benzo[*a*]pyrene, benzo[*a*]anthracene, and chrysene either indicated or, in some cases, proved that dihydrodiols on benzo rings with "bay-region" double bonds[7] are proximate carcinogens and that diol epoxides of these dihydrodiols are ultimate carcinogenic forms.[8] These results have led to the formulation of the bay-region theory, which postulates that epoxides which form part of a bay region on angular, saturated benzo rings, should have high chemical reactivity and biological activity for electronic reasons.[9] If this theory is applied to the substituted polycyclic aromatic hydrocarbon MC, the diol epoxide[10] predicted to have the highest chemical reactivity is that shown below:



Such a metabolite could form by hydration of MC 9,10-oxide to MC-9,10-dihydrodiol followed by epoxidation of the 7,8 double bond. To investigate this possibility, we have examined the oxidative metabolism of MC by the cytochrome P-450 dependent rat liver monooxygenase system.

In a preliminary study of the metabolism of [^{14}C]-MC by liver microsomes from immature, male Long-Evans rats,[6a] 1-hydroxy-MC (57%), 2-hydroxy-MC (16%), and 2-keto-MC (9%) were found to account for >80% of the total metabolites (4% total metabolism). Only trace amounts of metabolites (<3%) could be identified as dihydroxylated species, mainly *trans*-MC-11,12-dihydrodiol and 1,2-dihydroxy-MC. Similar product ratios were obtained for incubations with microsomes from MC-treated rats in which 25% of the substrate was metabolized.

In light of the somewhat surprising result that dihydrodiols represent only trace metabolites from MC, the possibility that 1-hydroxy-MC, the major primary oxidative metabolite of the hydrocarbon, might function as a dihydrodiol precursor was examined. Since the chromatographic mobilities of such 1-hydroxydihydrodiols were unknown, a highly purified monooxygenase system from rat liver[11] was used to study the metabolism of [1-^{3}H]-1-hydroxy-MC. Addition of homogeneous epoxide hydrase[12] to this system would result in the formation of dihydrodiols at the expense of phenols which are formed by isomerization of arene oxides. Such an experiment (Figure 1) revealed the presence of four dihydrodiols among the metabolites of 1-hydroxy-MC. These metabolites are not formed by the purifed monoxygenase system in the absence of epoxide hydrase (Figure 1).

To isolate sufficient amounts of these dihydrodiols for structure elucidation studies, liver microsomes were prepared from 200 immature, male Long-Evans rats which had been pretreated with MC to induce metabolism and were incubated with 0.51 mmol of racemic [1-^{3}H]-1-hydroxy-MC at 37 °C for 30 min.[13] In this study, 60% of the substrate was metabolized, and the two major dihydrodiol peaks (i.e., peaks a and b in Figure 1) accounted for 23 and 8% of the total metabolites which emerge from the column as distinct peaks.[14] Dihydrodiols a and b were isolated by preparative HPLC as follows: 200 μL of an 8-mL stock solution in THF was injected onto a

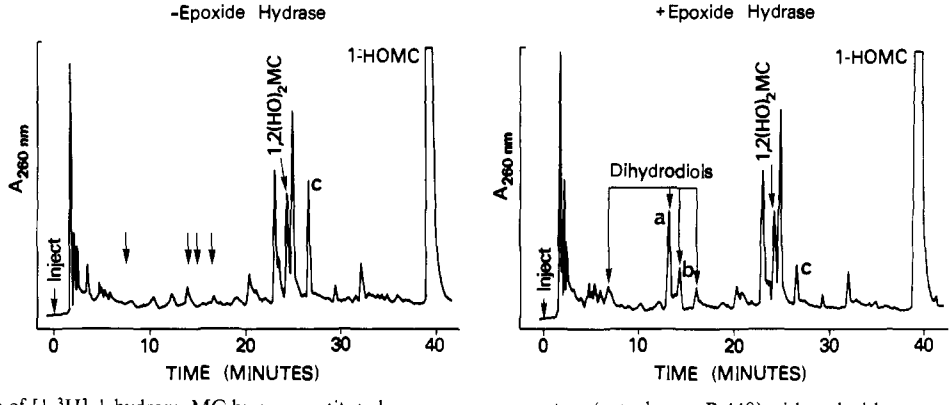


Figure 1. Metabolism of [1-^{3}H]-1-hydroxy-MC by a reconstituted monooxygenase system (cytochrome P-448) with and without epoxide hydrase. Analysis by HPLC was performed on a Du Pont Zorbax ODS column (6.2 mm × 25 cm) which was eluted with a linear gradient of 40–99% acetonitrile in water over a period of 59 min after a 1-min delay at a constant flow rate of 2.0 mL/min. [1-^{3}H]-1-Hydroxy-MC (8.4 μCi/μmol, 80 nmol in 0.1 mL acetone) was incubated with cytochrome P-448 (0.5 nmol), cytochrome *c* reductase (100 U), dilauryl phosphatidyl choline, phosphate buffer (200 μmol, pH 7.0), and $MgCl_2$ (6 μmol) in a total volume of 2 mL at 37 °C for 10 min. Incubations were performed either with or without epoxide hydrase (125 μg, 637 U/mg). The major dihydrodiols (peaks a and b) represented 10.5% of the total metabolites at 15% conversion of the substrate in the presence of epoxide hydrase. The dihydrodiols form mainly at the expense of the phenol containing peak c.

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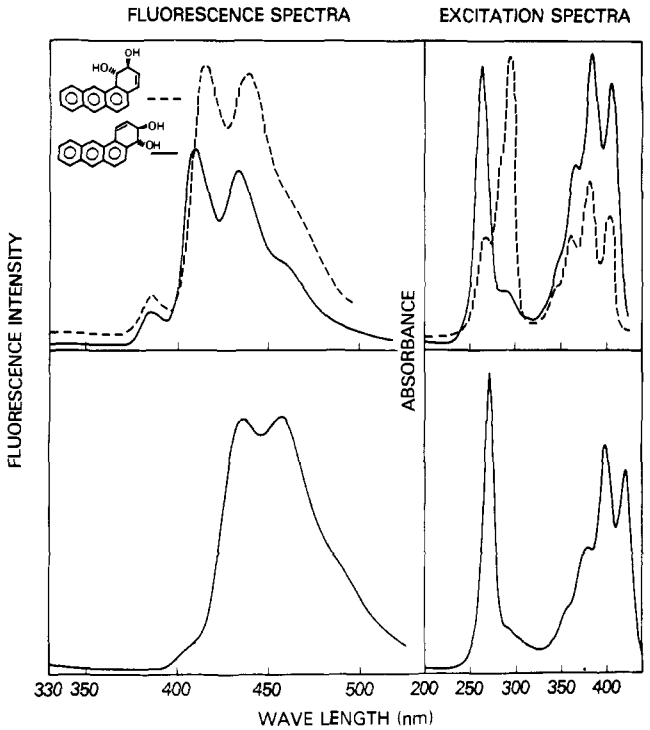
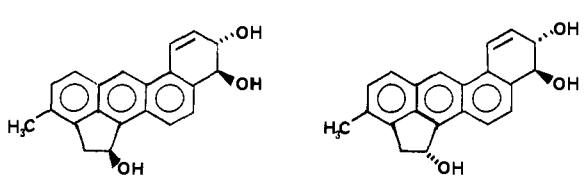


Figure 2. Fluorescence and excitation spectra of the 9,10-dihydrodiol a obtained from 1-hydroxy-MC. Fluorescence and excitation spectra of benzo[*a*]anthracene 1,2- and 3,4-dihydrodiols are shown for comparison and differ markedly from those of the 5,6-, 8,9-, and 10,11-dihydrodiols of benzo[*a*]anthracene.

Whatman Partsil-10 column (9.4 mm × 50 cm) which was eluted with 20% THF in CH_2Cl_2 at a flow rate of 24 mL/min. The two major dihydrodiols a and b eluted at retention times of 7 and 8 min, respectively. After isolation, each dihydrodiol was further purified by rechromatography with the same system to provide 5 mg of dihydrodiol a and 0.6 mg of dihydrodiol b.

Mass spectra of both metabolites (molecular ions at *m/e* 318 with M − H_2O peaks at 300) confirmed that these two metabolites are dihydrodiols of 1-hydroxy-MC. UV spectra of both metabolites (λ_{max} 268 nm (ϵ_{max} 72 600 M^{-1} cm^{-1})) were almost identical with that of *trans*-3,4-dihydroxy-3,4-dihydrobenzo[*a*]anthracene,[15a] indicative of a 1-vinylanthracene chromophore. Comparison of the fluorescence and excitation spectra of the metabolites with those of the 1,2- and 3,4-dihydrodiols of benzo[*a*]anthracene also indicated that both of the new metabolites have a 1-vinylanthracene chromophore (Figure 2). The ^1H NMR spectrum of the major dihydrodiol a (100 MHz, acetone) allowed assignment as *trans*-9,10-dihydroxy-9,10-dihydro-1-hydroxy MC: δ 7.44 (H_7), 6.25 (H_8), 4.65 (H_9), and 4.88 (H_{10}) with $J_{7,8}$ = 10, $J_{8,9}$ = 3, and $J_{9,10}$ = 10 Hz. The benzylic vinyl hydrogen H_7 is shifted downfield into the aromatic region due to edge deshielding of the aromatic system as expected for a bay-region double bond, and the coupling constants are those expected for a benzo-ring trans dihydrodiol which does not have the hydroxyl groups in a bay region.[15] Since racemic 1-hydroxy-MC was used in the incubation, dihydrodiols a and b (Figure 1) are presumed to be diastereomers in which the 1- and 10-hydroxyl groups are either cis or trans as shown below:



Work is in progress to elucidate the stereochemical fate of (+)- and (−)-1-hydroxy-MC on metabolism to its 9,10-dihydrodiols.

MC, 7-methylbenzo[*a*]anthracene, and 7,12-dimethylbenzo[*a*]anthracene are all alkyl-substituted, carcinogenic derivatives of benzo[*a*]anthracene. Studies with liver microsomal monooxygenase mediated activation of 7-methylbenzo[*a*]anthracene and its isomeric dihydrodiols to bacterial mutagens[16a] and to compounds which transform cells in culture[16b] have demonstrated that the 3,4-dihydrodiol with a bay-region double bond exhibits the highest activity. Studies with 7,12-dimethylbenzo[*a*]anthracene have shown that metabolism induced binding of the hydrocarbon to the DNA of cultured cells results in the adduct which has a 1,2,3,4-tetrahydro-7,12-dimethylbenzo[*a*]anthracene chromophore.[17] The bay-region diol epoxides (3,4-diol 1,2-epoxide) are presumably the active intermediates in both cases, and the data indicate that the bay-region theory[9] applies to the substituted as well as the unsubstituted polycyclic aromatic hydrocarbons. These studies, taken together with our preliminary finding that dihydrodiol a from 1-hydroxy-MC is metabolically activated to mutagens toward the *Salmonella typhimurium* test strain TA 100 to a 10-fold or greater extent than is MC, suggest that a bay-region diol epoxide of 1-hydroxy-MC may be an ultimate carcinogenic metabolite.[18] Although the present in vitro metabolism studies are novel in that they suggest that three oxidative steps (benzylic hydroxylation at C-1, dihydrodiol formation at the 9,10 positions, and epoxidation at the 7,8 positions) are important in the formation of an ultimate carcinogen from MC, the 9,10-diol 7,8-epoxides without the 1-hydroxyl group may also play an important role in the carcinogenicity of the hydrocarbon in vivo.

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(13) Each of 26 flasks contained racemic [1-^3H]-1-hydroxy-MC (20 μmol in 3.5 mL of acetone), liver microsomal protein (267 mg), phosphate buffer (pH 7.4, 5 mmol), $MgCl_2$ (150 μmol, NADP (50 μmol), glucose 6-phosphate (250 μmol), and glucose 6-phosphate dehydrogenase (70 U) in a total volume of 50 mL. Products and unreacted substrate were extracted from the pooled incubation medium with ethyl acetate:acetone (2:1, 150 mL), and the organic layer was separated, dried (anhydrous Na_2SO_4), and evaporated.
(14) These percentages fall to 5 and 2% if the total radioactivity which emerges

from the column prior to the substrate is considered. This results from additional readiactive metabolites which do not chromatograph as discrete peaks but cause a general increase in the base line radioactivity throughout the chromatographic profile.
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(18) Full details of the metabolic activation studies will be published elsewhere.
(19) Authors are from (a) National Institutes of Health, (b) Hoffmann-La Roche, Inc., and (c) Medical College of Georgia. T.A.S. acknowledges the financial assistance from NCI Grant CA 21481.

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Crystal Structure of Bis(η^5-cyclopentadienyl)-chloro(neopentylidene)tantalum, Ta(η^5-C_5H_5)$_2$(CHCMe$_3$)-Cl, a Molecule Containing a Twisted Neopentylidene Ligand with a Highly Obtuse Ta–C(α)–C(β) Angle

Sir:

The only x-ray structural study of a primary alkylidene[1] complex, Ta(η^5-C_5H_5)$_2$(CH_2)(CH_3),[2] showed that the Ta=CH_2 bond was short (Ta=CH_2 = 2.026 (10) Å as opposed to Ta—CH_3 = 2.246 (12) Å) and that the planar CH_2 ligand took up an orientation perpendicular to the C–Ta–C plane, the dihedral angle between CH_2 and C–Ta–C systems being 88 (3)°.[2] ^{1}H NMR studies were consistent with these findings and demonstrated, in addition, that the barrier ($\Delta G^{\ddagger}_{rot}$) to the methylene ligand turning by 90° about its Ta–C vector (i.e., "rotation") was ≥21 kcal/mol in the closely related asymmetric species Ta(η^5-C_5H_5)(η^5-C_5H_4Me)(CH_2)(CH_3). $\Delta G^{\ddagger}_{rot}$ for Ta(η^5-C_5H_5)$_2$(CHCMe$_3$)Cl[3] was, however, substantially smaller (16.8 kcal/mol at 323 K).[2] A single-crystal x-ray structure analysis of Ta(η^5-C_5H_5)$_2$(CHCMe$_3$)Cl has now been undertaken in order to provide details as to how the neopentylidene ligand is bound and, in particular, to see if any ligands in the ground-state structure are distorted significantly (compared with those for Ta(η^5-C_5H_5)$_2$(CH_2)(CH_3)) in a manner which would account for a significantly lower barrier to apparent rotation of the alkylidene ligand.

The complex crystallizes from acetonitrile in the centrosymmetric monoclinic space group $P2_1/c$ with a = 6.5957 (8) Å, b = 15.4418 (19) Å, c = 14.3363 (19) Å, β = 103.023 (10)°, V = 1422.6 (3) Å^3, Z = 4, and ρ(calcd) = 1.946 g cm^{-3} for mol wt 416.73. Intensity data were collected via θ–2θ scans with a Syntex $P2_1$ automated diffractometer[4] and were corrected for absorption (μ = 77.6 cm^{-1}) by an empirical method based upon a series of ψ scans. The structure was solved via Patterson and difference-Fourier methods; full-matrix least-squares refinement (Ta, Cl, and C anisotropic; H isotropic) led to final discrepancy indices R_F 2.7% and R_{wF} 2.3% for all 1870 unique reflections (*none rejected*) in the range 4° ≤ 2θ ≤ 45° (Mo Kα radiation). All atoms, including all hydrogen atoms, have been located and refined; the molecular geometry is shown in Figure 1.

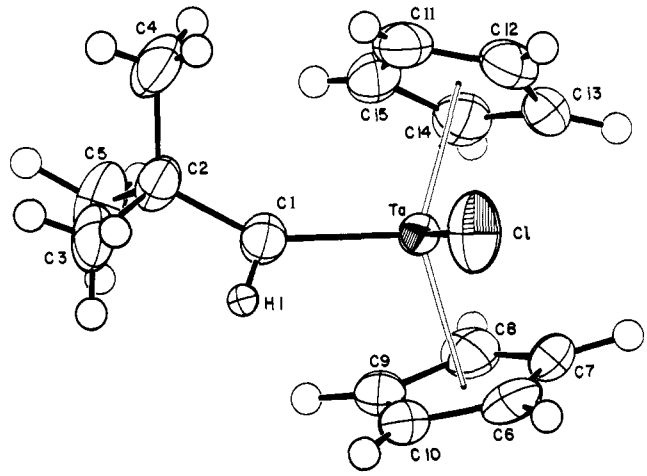

Figure 1. Geometry and labeling of atoms in the Ta(η^5-C_5H_5)$_2$-(CHCMe$_3$)Cl molecule (ORTEP2 diagram; 50% ellipsoids for all nonhydrogen atoms, with thermal spheres of hydrogen atoms artificially reduced).

Several features are entirely as expected. The centroids of the planar, eclipsed η^5-C_5H_5 rings subtend an angle of 130.9° at the metal and the C(1)–Ta–Cl angle is 97.63 (18)°. The Ta–Cl distance is 2.479 (2) Å and the C(1)–C(2) and C(1)–H(1) distances are 1.523 (9) and 0.82 (6) Å, respectively. C(2)–CH_3 distances range from 1.514 (10) to 1.544 (10) Å, while C–H(Me) distances lie in the range 0.77 (7)–1.08 (8) Å (average 0.94 Å).

Atom C(1) of the neopentylidene ligand, which is essentially coplanar with Ta, C(2), and H(1), is bound to Ta through what is believed to be essentially a double bond (Ta–C(1) = 2.030 (6) Å; cf. 2.026 (10) Å in the Ta=CH_2 complex). It is ~0.22 Å shorter than a single bond.

The two unusual and surprising features of the molecular geometry are as follows. (1) ∠(Ta–C(1)–C(2)) is extraordinarily obtuse for an angle at a formally sp^2-hybridized carbon atom, having a value of 150.4 (5)°; the remaining angles at C(1) are correspondingly reduced, i.e., ∠(Ta–C(1)–H(1)) = 111 (4) and ∠(C(2)–C(1)–H(1)) = 99 (4)°. (2) The dihedral angle between the Cl–Ta–C(1) and Ta–C(1)–C(2) planes is only 79.7°—i.e., the neopentylidene moiety is displaced by ~10.3° from the ideal perpendicular geometry. The first of these features could result (at least in part) from steric interaction between the –CMe_3 group and an η^5-C_5H_5 ligand. The second almost certainly does; we feel that further rotation of $CHCMe_3$ into the C–Ta–Cl plane therefore is easier when the methylene ligand is substituted.

We can draw two conclusions from the available data. These are as follows. (1) Since $\Delta G^{\ddagger}_{rot}$ decreases markedly when θ (the deviation of the =CHR plane from the perpendicular) increases to only ~10°, it follows that the π orbital on C(α) does not overlap well with the π orbital on Ta. (2) Since the methylene C(α) in Ta(η^5-C_5H_5)$_2$(CH_2)(CH_3) is demonstrably nucleophilic, the transition state for rotation about the Ta–C(α) bond—where the $CHCMe_3$ ligand has rotated into the C(α)–Ta–Cl plane of Ta(η^5-C_5H_5)$_2$(CHCMe$_3$)Cl—is probably best described in valence bond terms as the 1,2-dipolar form, (η^5-C_5H_5)$_2$(Cl)Ta$^+$–$^-$CHCMe$_3$, in which tantalum is in a valence state of +5 (d^0). The usual description of M=CHR bonding as composed of π donation from a filled sp^2 orbital on C(α), coupled with back-donation from a filled metal π orbital into an empty 2p$_z$ orbital on C(α), is probably not correct for the bonding of an alkylidene ligand to a fairly electropositive metal atom.[5] Rather, the π *bond* is better re-

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