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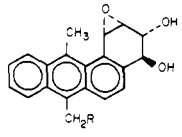
Synthesis of *trans*-3,4-Dihydroxy-3,4-dihydro-7,12-dimethylbenz[*a*]anthracene, a Highly Carcinogenic Metabolite of 7,12-Dimethylbenz[*a*]anthracene

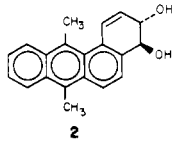
Sir:

7,12-Dimethylbenz[*a*]anthracene (DMBA) is one of the most potent known carcinogenic hydrocarbons,[1,2] and DMBA-induced tumors are widely employed in cancer research. Recent studies have implicated the diol epoxide *trans*-3,4-dihydroxy-*anti*-1,2-epoxy-1,2,3,4-tetrahydro-



1a : R = H
b : R = OH

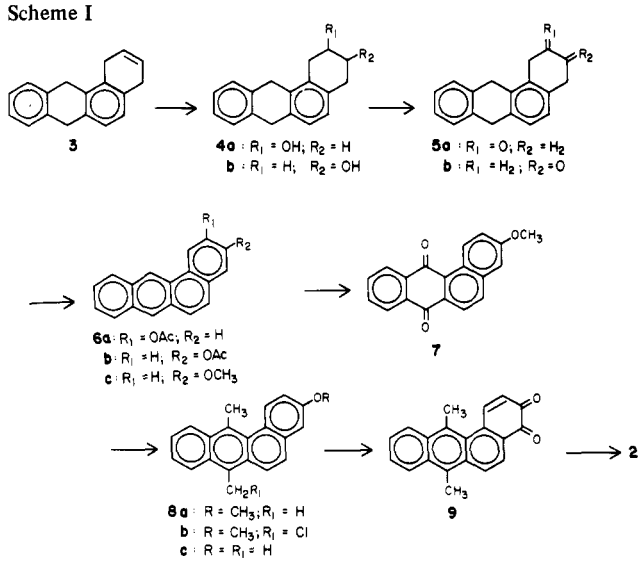
DMBA (**1a**)[3,4] and/or the related triol epoxide **1b**[5] as the principal metabolically activated form(s) of DMBA which bind covalently to DNA in vivo.[6] The structures of these hypothetical intermediates were tentatively assigned on the basis of UV fluorescence and chromatographic data on the nucleic acid bound metabolites. Consistent with the hypothesis that **1a** and/or **1b** are the active carcinogenic forms of DMBA, the 3,4-dihydrodiol of DMBA (**2**), thought to be the metabolic precursor of **1a**, is found to undergo microsome-mediated transformation to a highly mutagenic intermediate presumed to be **1a**.[11] These findings are consistent with other evidence implicating diol epoxide metabolites as the principal (though not the sole)[9] active forms of other carcinogenic hydrocarbons.[7] Although synthesis of the 8,9,10,11-diol epoxide of DMBA has been described,[12] synthesis of **1a,b** remains a challenging objective.[13]



2

We now report efficient synthesis of **2** via a novel route. Attempts to adapt the synthetic approaches devised for the analogous dihydrodiols of unsubstituted polycyclic arenes[7a] were unsuccessful, principally owing to competitive side reactions on the reactive methyl groups. The method eventually devised (Scheme I) is potentially applicable with appropriate

Scheme I



modification to the synthesis of many presently difficultly accessible analogous derivatives of other potent carcinogenic hydrocarbons (e.g., 7-methylbenz[*a*]anthracene and 3-methylcholanthrene).

Reduction of benz[*a*]anthracene (BA) with lithium in ammonia according to the published procedure[14] gave in two steps 1,4,7,12-tetrahydro-BA (**3**). The latter underwent reaction with diborane and alkaline H_2O_2[15] to a mixture of 2- and 3-hydroxy-1,2,3,4,7,12-hexahydro-BA (**4**).[16] Oxidation of the mixed alcohols with trifluoroacetic anhydride and Me_2SO according to the method of Swern[17] gave the mixed ketones **5a,b** where were separated by chromatography on Florisil. 3-Oxo-1,2,3,4,7,12-tetrahydro-BA (**5b**) underwent efficient conversion to 3-acetoxy-BA (**6b**) via formation of the enol acetate and dehydrogenation with *o*-chloranil by conventional procedures; methylation with dimethyl sulfate and sodium methoxide in methanol gave 3-methoxy-BA (**6c**).[18] 2-Acetoxy-BA (**6a**)[19] was obtained from the 2-keto compound **5a** through an analogous sequence of transformations. The synthesis of **6a–c** described constitute the first syntheses of A-ring substituted derivatives of BA directly from the parent hydrocarbon.[20]

Conversion of 3-methoxy-BA to 3-methoxy-DMBA (**8a**) was accomplished in high overall yield through initial oxidation of **6c** with sodium dichromate in acetic acid to the quinone **7**.[21] The latter underwent transformation to **8a** through reaction with methyllithium, followed by treatment with gaseous HCl by Newman's method[22] and reduction of the resulting 7-chloromethyl intermediate **8b** with $NaBH_4$.[23] Protection of the phenolic group as the methyl ether during reaction with methyllithium was found necessary, since attempted similar reactions on the free phenol or the acetate ester gave markedly diminished yields. Demethylation with lithium thiomethoxide[24] furnished the free phenol **8c** in 95% yield: mp 169 °C (benzene–hexane); NMR ($CHCl_3$) δ 3.05 (s, 3 H, 7-CH_3), 3.30 (s, 3 H, 12-CH_3), 7.0–8.5 ppm (m, 9 H, aromatic).

Oxidation of the phenol **8c** with Barton's reagent (benzeneselenic anhydride)[25] gave dark violet crystals of DMBA-3,4-dione (**9**) (80%), mp 181–183 °C dec. Compound **9** is apparently the first terminal ring *o*-quinone derived from a carcinogenic polyarene.[26] Reduction of **9** with $LiAlH_4$ afforded the *trans*-3,4-dihydrodiol **2**, mp 182–184 °C. The yield of **2** (43%) compares quite favorably with the 1–5% yields reported for analogous reductions of anthracene-1,2-dione and the phenanthrene-1,2- and -3,4-diones;[27] the improvement is at least partially a consequence of avoidance of acidic conditions during workup.

The integrated 270-MHz NMR spectrum of **2** was entirely

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consistent with the assigned structure; the observed chemical shifts and coupling constants were also in agreement with the NMR data reported by Tierney et al.[13] following completion of these studies. The H_1 signal appeared at low field consistent with its assigned bay region location. The H_2 proton was found as a doublet of doublets at δ 6.13 coupled to H_1 and H_3 ($J_{1,2}$ = 9.5, $J_{2,3} \simeq$ 2.2 Hz). The two carbinol H_3 and H_4 peaks overlapped at δ 4.60 and 4.69, respectively. The relatively large value of $J_{3,4}$ ($J_{2,3}$ = 2.3, $J_{3,4}$ = 11.5 Hz) confirms the trans stereochemical relationship of the hydroxyl groups and indicates that this dihydrodiol exists in solution predominantly in the trans-diequatorial conformation;[28] much smaller coupling constants are expected for the cis isomer or for the trans-diaxial conformation.[7d,28]

Investigation of the carcinogenic activity of **2** has revealed it to be *more potent than DMBA and the most carcinogenic hydrocarbon metabolite tested to date.*[29] In comparison with the 5,6- and 8,9-dihydrodiols of DMBA, **2** (100 nmol) was found to induce tumors in 29/29 surviving mice (22.8 papillomas/mouse), while the other two dihydrodiols were essentially inactive. At lower dosage (10 nmol) **2** still induced tumors in 100% of mice (15.2 papillomas/mouse), whereas DMBA gave 85% tumor induction (4.8 papillomas/mouse). This is strong evidence for the intermediacy of **2** as a proximate carcinogenic metabolite and **1a** (and/or **1b**) as the ultimate carcinogenic form of DMBA.

The generality of the synthetic method depicted in Scheme I is supported by studies in progress aimed at extension of the method to the analogous dihydrodiols of other polycyclic arenes. *trans*-3,4-Dihydroxy-3,4-dihydro-7-methyl-BA, implicated as the proximate carcinogenic form of 7-methyl-BA,[30] has been synthesized successfully in our laboratory via a related synthetic sequence starting with 7-methyl-BA and eliminating the steps **6** → **7** → **8** involved with introduction of the methyl groups. Full details of this and other related syntheses will be reported in due course.

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Note Added in Proof. Since submission of this manuscript **2** has been characterized as a metabolite of DMBA by S. K. Yang, M. W. Chou, and P. P. Roller, *J. Am. Chem. Soc.*, **79,** 237 (1979); NMR data reported therein are in good agreement with those observed for authentic **2** obtained through synthesis.

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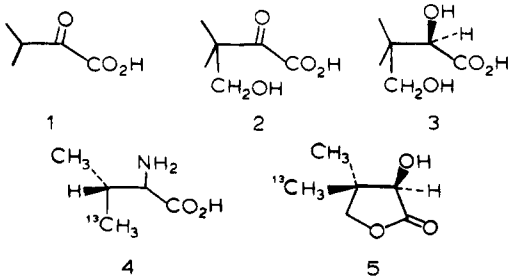
Stereochemistry of Pantoate Biosynthesis from 2-Ketoisovalerate

Sir:

The biosynthesis of D-pantoate **3** constitutes the first stage in the formation of pantothenate, which is ultimately utilized in the biosynthesis of the acyl group carriers, coenzyme A and acyl carrier protein.[1] The first step in pantoate biosynthesis is the reaction of 2-ketoisovalerate **1** with N^5,N^{10}-methylenetetrahydrofolate to yield 2-ketopantoate, **2.** The enzyme, ketopantoate hydroxymethyltransferase (5,10-methylenetetrahydrofolate:α-ketoisovalerate hydroxymethyltransferase), was recently isolated from *E. coli* and characterized by Snell and co-workers.[2] Subsequently, 2-ketopantoate is reduced by 2-ketopantoate reductase[3] to D-pantoate **3.** I now present ev-



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