$KHSO_4 \cdot K_2SO_4$, 46.0 g) in 225 mL of water was added dropwise over a period of 1.5 h. The pH was maintained at 7.5–8.5 by monitoring with a pH electrode and dropwise addition of an aqueous solution of KOH (2–3%). The reaction mixture was stirred for an additional 4 h and then mixed with an equal volume of ice cold water. The reaction mixture was then extracted with methylene chloride and the extract washed three times with ice-cold water. The methylene chloride layer was dried (K_2CO_3) and the solvent removed on the rotary evaporator. TLC analysis of the solid residue indicated that it contained only phenanthrene and phenanthrene oxide. The arene oxide was isolated by preparative TLC with methylene chloride/hexane (1:1) as the eluent. The product was recrystallized from methylene chloride/hexane (1:1) to give shining white flakes of the oxide. Oxide obtained in this manner gave two separate melting points, 127–128 °C and 145–147 °C. Similar melting point behavior has been reported in the literature, i.e., 124–125[15] and 148 °C.[16] The results obtained with a series of arenes are shown in Table I. All of the oxides listed in Table I had melting point and spectral data that were identical with those given in the literature or with those of samples prepared locally[17] by using literature methods. Control reactions using caroate alone showed that no oxide is obtained in the absence of acetone except in the cases of **3** and **4** where traces of oxide were observed. Similarly when the procedure is attempted with methanol or dioxane instead of acetone no oxide is formed in the phenanthrene case.

Arene oxides have been successfully synthesized by several routes.[18–34] The simplest of these appears to be the use of hypochlorite as described by Hamilton.[18] Use of the caroate–acetone system may be a competitive synthetic method. Work in progress is aimed at determining the requirements, particularly the electronic requirements, for O atom transfer by dioxiranes. It should be noted that the yield data for arenes **3** and **4** contain a hint that the transfer reaction may be electrophilic in nature.

We find the evidence[5–9] to be convincing that the caroate–acetone system involves the formation of dioxiranes. In earlier studies we[35–38] and Jerina et al.[39,40] have described cases where O atom transfer to arenes has been attributed to carbonyl oxides. The present results which we believe are best interpreted as involving O atom transfer from dimethyldioxirane, raise questions about the involvement of dioxiranes in a range of organic oxidations involving peroxides including those in which a carbonyl oxide is the presumed oxidant.[41]

It has been demonstrated[1–3] that gas-phase ozonolyses can produce dioxiranes. Urban atmospheres frequently contain relatively high concentrations of ozone, olefins, and PAH. Such atmospheres thus contain the essential elements for the production of dioxiranes and/or carbonyl oxides and it seems likely that these reactive intermediates would undergo subsequent reactions with PAH to give arene oxides. Indeed we have been able to show[43] that a carbonyl oxide produced via ozonolysis of a suitable PAH absorbed on a model particulate surface (silica gel) leads to the formation of a K-region oxide by intramolecular O atom transfer. It is now well established that carcinogenic PAH require metabolic activation, i.e., oxidation, prior to displaying ultimate carcinogenicity. Such metabolic activation produces arene oxides and other metabolites. Production of arene oxides in polluted atmospheres, as postulated here, would thus increase the negative impact of such atmospheres on environmental health.

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Shikimate-Derived Metabolites. 13. A Key Intermediate in the Biosynthesis of Anthranilate from Chorismate

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It has been established in microorganisms that chorismic acid (**1**) is the precursor of anthranilic acid (**4**), a key metabolic intermediate in tryptophan biosynthesis.[1] Although much is known about anthranilate synthase (AS),[2] the enzyme that transforms **1** into **4** using ammonia or glutamine, the step-by-step chemical events mediated by the protein remain obscure. In 1962, McCormick et al. reported that amino alcohol **2** was produced by a strain of *Streptomyces aureofaciens*,[3,4] prompting the suggestion[5] that trans enol pyruvate **3** was a likely intermediate between **1** and **4** (drawings denote absolute stereochemistry). Since then, several efforts to isolate and characterize this species have failed,[6] and even its proposed structure remains controversial.[1]

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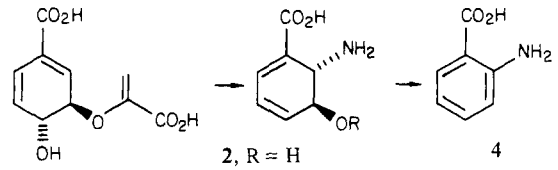
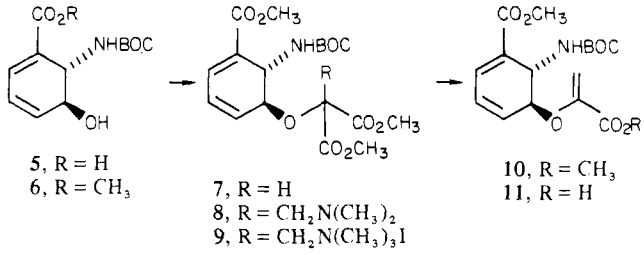
In view of recent work in this area,[7] we now describe an unambiguous synthesis of (+)-*trans*-6(*S*)-amino-5(*S*)-[(1-carboxyethenyl)oxy]-1,3-cyclohexadiene-1-carboxylic acid (**3**) and the characteristics of its enzyme-catalyzed conversion to **4**.



1 2, R = H 3, R = $C(CH_2)CO_2H$ 4

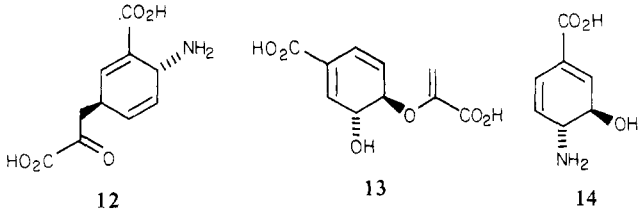
A sample of 2(*S*),3(*S*)-dihydro-3-hydroxyanthranilic acid (**2**) ($[\alpha]_D$ +445°, generously provided by Dr. J. R. D. McCormick of Lederle Laboratories) was smoothly N-protected as its BOC derivative **5** (BOC-ON™, dioxane/H_2O/Et_3N) then carefully esterified with diazomethane (ether–CH_3OH, 0 °C) to afford **6** in 67% overall yield. Using methodology we developed for the construction of enol pyruvates,[8] we transformed **6** to alkoxymalonate **7** in 56% yield upon exposure to dimethyl diazomalonate (2.3 equiv) in the presence of a catalytic amount of Rh_2OAc_4 (C_6H_6, 80 °C, 1 h). Condensation of **7** with Eschenmoser's salt [CH_2=$N(CH_3)_2I$, CH_2Cl_2, Et_3N, 93%] formed Mannich base **8**, which without purification was quaternized immediately to **9** (CH_3I, CH_2Cl_2, 97%). Fragmentation of **9** in NaOH (CH_3OH–H_2O, 0 °C, 2 h) afforded dimethyl ester **10** in 67% yield. This



5, R = H
6, R = CH_3

7, R = H
8, R = $CH_2N(CH_3)_2$
9, R = $CH_2N(CH_3)_3I$

10, R = CH_3
11, R = H



12 13 14

substance could be saponified (2.9 equiv of NaOH, THF–H_2O, 0 °C, 3.5 h, 92%) and the resulting diacid **11** freed of some minor aromatic impurities by silica gel chromatography. Deprotection of **11** in trifluoroacetic acid (neat TFA, 0 °C, 15 min, 60%) furnished the desired (*S*),(*S*)-*3* in pure form as its TFA salt: $[\alpha]_D^{22}$ +205° (*c* 1.6, H_2O), mp 94–96 °C (lit.[7] mp 93–95 °C, racemate). This salt proved to be rather unstable, even when stored at −20 °C. NMR monitoring of a D_2O solution of **3**·TFA indicated complete conversion to **12** (by Claisen rearrangement) plus unidentified aromatic material(s) within 20 h at room temperature.

Anthranilate synthase from enteric bacteria usually consists of two dissimilar subunits designated AS-I and AS-II. While the native enzyme will utilize either glutamine or NH_4^+ to transform **1** into **4**, AS-I by itself requires NH_4^+ and cannot use glutamine. Pyruvate is also produced, but does not incorporate the C2 hydrogen of **1**.[9] By standard assay conditions at pH 8.6,[2] freshly prepared (within 12 h) (+)-**3**·TFA salt was tested as a potential intermediate in the enzymatic biosynthesis of anthranilate using pure AS-I from *Serratia marescens*.[10]

We found that the proposed intermediate, with absolute configuration as designated, was indeed an effective substrate for AS-I. In the absence of NH_4^+, (+)-**3**·TFA was converted to anthranilate with a V_{max} of 120 (nmol/min)/mg. When 50 mM $(NH_4)_2SO_4$ was added to the medium, V_{max} dramatically increased to 610 (nmol/min)/mg (K_M = 0.1 mM). By comparison, chorismate and NH_4^+ formed anthranilate with a V_{max} of 500 (nmol/min)/mg (K_M = 0.1 mM).[2,11] These rates thus establish (+)-**3** as a kinetically viable biosynthetic intermediate. A group at MIT recently showed that synthetic, racemic **3** was converted to **4** in the presence of NH_4^+ with $V_{max} \approx 600$ (nmol/min)/mg and K_M = 0.2 mM.[7,12] The discrepancy between the two K_M values is consistent with expected biological enantioselectivity.

It was of some concern that enzymatic yields of anthranilate from (+)-**3** were unusually poor (21–37% by fluorescence assay) and could not be improved by adding more enzyme. Policastro et al. noted (but did not explain) a similar phenomenon.[7] The following experiments indicate that both substrate breakdown and enzymic inhibition contributed to the low conversions. (1) Eight-hour-old, buffered solutions of (+)-**3**·TFA were reassayed with AS-I, and the observed turnovers were 22–32% lower than those obtained with freshly prepared solutions. Controls showed no such effect with chorismic acid. (2) If fresh chorismate was added to 8-h-old incubations of (+)-**3** with AS-I, no additional **4** whatsoever was produced. Furthermore, in those same incubations, anthranilate production was restored to only 10% of its expected level when both fresh chorismate and enzyme were added. (3) Powerful enzymic inhibition (or inactivation) was evident when solutions of **1** and (+)-**3**, now 8 h old in buffer, were mixed and assayed by enzyme. At several different concentrations, measured rates of anthranilate formation fell far short of the expected levels.

One obvious candidate for the inhibitor is rearrangement product **12**, which, judging from molecular models, may function as a substrate or transition-state analogue. Although we have not yet obtained a pure sample of **12**, chromatography fractions of (+)-**3** containing **12**, *when freshly dissolved in buffer*, did exhibit the expected degree of enzymic inhibition.

Because of growing interest in the regulation of tryptophan biosynthesis, the effect of several other substances on AS-I was also evaluated: pseudochorismic acid (**13**),[8] amino alcohol (*S*),(*S*)-**2**, lacking an enol pyruvate side chain,[13] and *trans*-amino alcohol ±**14**, synthesized earlier in these laboratories as part of an effort to study the biosynthesis of *p*-aminobenzoic acid (PABA) from **1**.[14] (Compound **14** was tested in view of recent biochemical and immunological studies suggesting a close structural homology and evolutionary relationship between anthranilate and PABA synthase components.[15]) None of these structures had any effect as substrates or inhibitors on AS-I. However *m*-hydroxybenzoic acid proved to be a modest inhibitor of this enzyme, with K_i = 0.03 mM. Further studies on the chemistry and biochemistry of **12** are in progress.

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