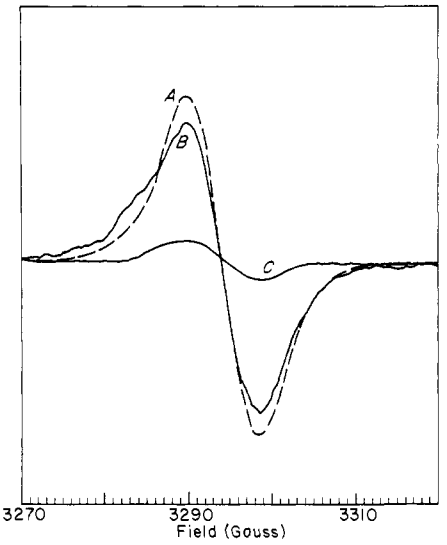

Figure 1. EPR spectra resulting from incubation of chromate (1.9 mM) with rat liver microsomes from phenobarbital-treated animals (11.6 mg/mL) and NADPH (0.88 mM) in 0.05 M Tris·HCl, pH 7.4 at 22 °C for (A) 0.5 min or (B) 10 min. (C) EPR spectrum resulting from incubation of chromate (1.9 mM) with NADPH (0.88 mM) in 0.05 M Tris·HCl, pH 7.4, at 22 °C for 0.5 min. Spectra were run on a Varian E-9 spectrometer at 77 K, 100-KHz modulation frequency, 3.2-G modulation amplitude, 100-μW microwave power, 9.124-GHz microwave frequency, and 6.3×10^3 (B), (C), or 2.5×10^3 gain (A).

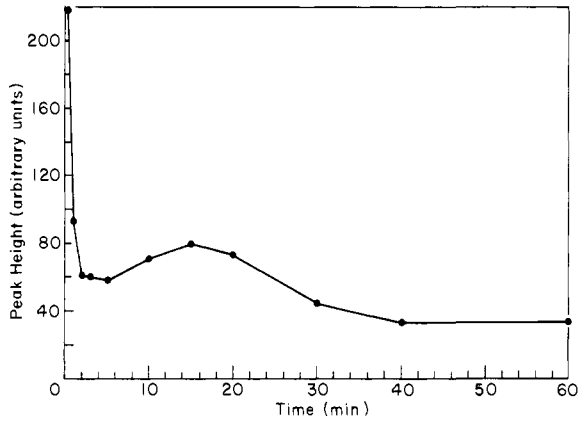

Figure 2. Time course for the appearance and loss of the EPR signal generated upon incubation of chromate, microsomes, and NADPH in 0.05 M Tris·HCl, pH 7.4, at 22 °C. Conditions were as described in Figure 1.

chromate is a likely mechanism for the rapid formation of chromium(V). Chromium(V) complexes are generally characterized as being labile and reactive, whereas chromium(III) complexes are substitution inert.[11] The fact that these chromium(V) intermediates persist for over 1 h in vitro make them likely candidates for the "ultimate" carcinogenic forms of carcinogenic chromium compounds.

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Registry No. Cr(V), 14280-17-2; chromate, 11104-59-9.

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Intermediates in the Reaction of Catechol 1,2-Dioxygenase with Pyrogallol and Oxygen

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The characterization of transient oxygenated complexes has provided valuable insights into the mechanisms of oxygenase-catalyzed reactions.[1-3] In the case of the catechol dioxygenases,[4] oxygenated intermediates have been reported for protocatechuate 3,4-dioxygenase.[5-8] Optical spectra of these intermediates generated with slow substrates can be observed under steady-state conditions; they exhibit absorbance maxima near 520 nm,[6] similar to those of carboxylate inhibitor complexes.[9] These spectra result from perturbations of phenolate-to-Fe(III) charge-transfer interactions characteristic of these enzymes.[9-12] Quenching experiments, among others, suggest that these intermediates may be enzyme-product complexes.[9,13,14] In reexamining the stopped-flow kinetics of protocatechuate 3,4-dioxygenase with protocatechuate and oxygen, Ballou and Bull[7] have discovered two intermediates, neither of which resemble that reported by Fujisawa et al.[5] In this communication, we report the observation of two oxygenated intermediates in the reaction of catechol 1,2-dioxygenase with pyrogallol and oxygen. These "snapshots" along the mechanistic pathway provide a further understanding of how these dioxygenases effect the catalysis of ring-cleavage reactions.

Pyrogallol is a slow substrate of catechol 1,2-dioxygenase[15] with a turnover number of 0.1 s^{-1} compared to 25 s^{-1} for catechol at 25 °C in potassium phosphate buffer, pH 7.5. Nozaki has reported that the reaction of catechol 1,2-dioxygenase with pyrogallol and oxygen results in two organic products, 2-pyrone-6-carboxylic acid and α-hydroxy-*cis,cis*-muconic acid.[16] When catechol 1,2-dioxygenase in potassium phosphate buffer, pH 7.5, is treated with pyrogallol at 1 °C in the presence of air, a spectrum which differs from those of the native enzyme and the enzyme–pyrogallol complex is obtained under steady-state conditions (Figure 1). The steady-state intermediate then decays to the enzyme–substrate complex when oxygen is depleted. The EPR spectrum of this

† National Institutes of Health Predoctoral Trainee, 1980–1982.

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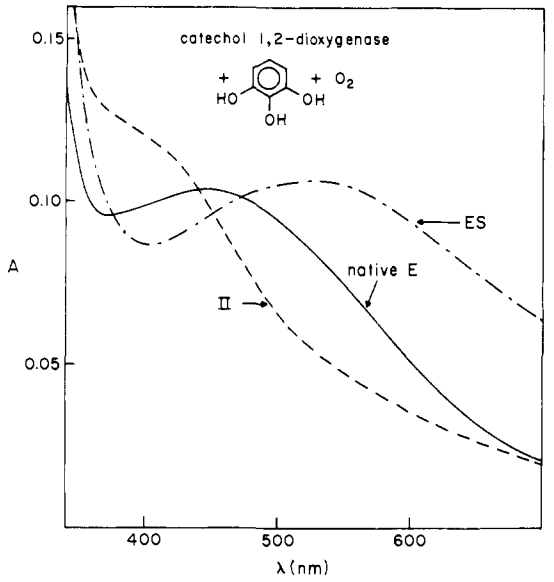

Figure 1. Visible spectra of native catechol 1,2-dioxygenase, its complex with pyrogallol, and the steady-state intermediate derived from enzyme, pyrogallol, and O_2. Conditions: 2.0 mg/mL enzyme, 1 mM pyrogallol, atmospheric O_2, 1 °C, 50 mM potassium phosphate buffer, pH 7.5. Spectra were obtained on a Cary 219 spectrophotometer.

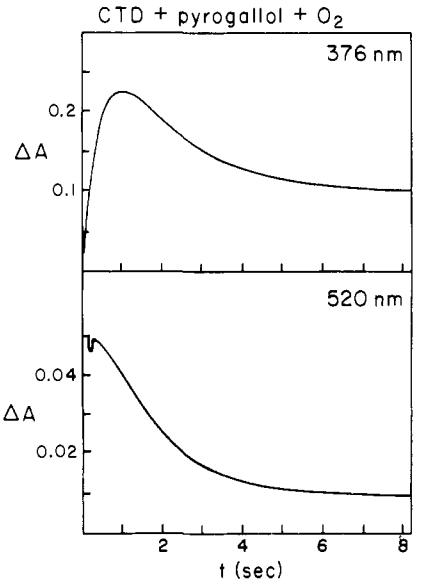

Figure 2. Stopped-flow kinetic traces of the reaction of catechol 1,2-dioxygenase with pyrogallol and O_2 at 376 and 520 nm at 17 °C. Aerobic enzyme solution (3.2 mg/mL) was mixed with anaerobic pyrogallol solution (2 mM) in 50 mM potassium phosphate buffer. Data were obtained on Durrum Instruments rapid kinetics spectrophotometer.

intermediate shows signals near $g = 9$ and 4.3, typical of high-spin ferric centers in rhombic symmetry.[17] Mössbauer spectra of this intermediate at liquid helium temperatures reveal the generation of a new species (ca. 80%) distinct from the native enzyme and the ES complex.[18] This species exhibits magnetic hyperfine interactions characteristic of a high-spin ferric complex.[19]

Stopped-flow kinetic studies (Figure 2) at 17 °C reveal two first-order processes. At 376 nm, an isosbestic point between E and ES, we observe an initial fast rise in absorbance ($k = 5.8\ s^{-1}$) followed by a slower decrease ($k = 0.53\ s^{-1}$). At 520 nm, the first process is manifested as a delay prior to the onset of the second process ($k = 0.54\ s^{-1}$) which corresponds to the formation of the steady-state intermediate. Two intermediates (labeled I and II) are thus observed in the reaction of catechol 1,2-dioxygenase with pyrogallol and O_2. The visible spectrum of intermediate I is approximated by that shown in Figure 3, which was obtained on a rapid scan spectrophotometer approximately 2 s after the components were mixed; after a few seconds, this decays to the spectrum of II.

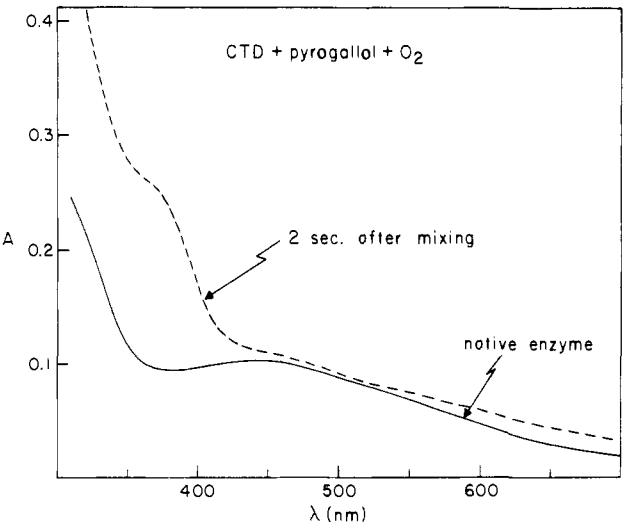

Figure 3. Visible spectra of native catechol 1,2-dioxygenase and intermediate(s) observed approximately 2 s after mixing enzyme, substrate, and O_2 at ca. 10 °C. Component concentrations as in Figure 1. Spectra obtained on a HP8450A rapid scan spectrophotometer.

The visible spectra of the two intermediates differ markedly from those of all other complexes of catechol 1,2-dioxygenase studied thus far.[11] The visible spectrum of II, however, closely resembles that of the intermediate labeled ESO_2 observed by Bull et al.[8] in stopped-flow experiments with protocatechuate 3,4-dioxygenase, protocatechuate, and oxygen. The similarity of these two intermediates serves to emphasize the point that similar mechanistic pathways are utilized by the two dioxygenases. Yet the kinetics observed in these two cases are clearly different; while ESO_2 is the first species observed for protocatechuate 3,4-dioxygenase, intermediate II is the second observed in the catechol 1,2-dioxygenase sequence. Intermediate I thus represents yet another catalytically significant species in the dioxygenase reaction cycle.

The chemical nature of these intermediates remains to be elucidated. Both intermediates exhibit visible spectra, suggesting that the phenolate-to-Fe(III) charge-transfer interaction persists in these complexes. This is corroborated by our EPR and Mössbauer results on intermediate II which unequivocally demonstrate that this complex has a high-spin ferric center. Since II closely resembles the ESO_2 complex of protocatechuate 3,4-dioxygenase, ESO_2 by inference is also a high-spin ferric complex, in contradiction to the suggestion that ESO_2 may be a ferrous semiquinone complex.[8] ESO_2 is observed to proceed to ESO_2* and then to EP in the protocatechuate 3,4-dioxygenase sequence,[8] so I and II must correspond to species closer to ES than to EP in the dioxygenase mechanism. We have proposed a mechanism[20] wherein a ferric catecholate complex reacts with oxygen to yield sequentially (a) a ferric semiquinone complex and superoxide, (b) a ferric peroxide complex, (c) a ferric hydroxide complex and *cis,cis*-muconic anhydride, and (d) a ferric carboxylate (product) complex. The assignment of I and II to any of the above species will have to await further experiments on these complexes. When these "snapshots" of the catalytic process are fully characterized, we should be able to assemble a coherent picture of enzyme-catalyzed catechol cleavage.

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Acknowledgment. This work was supported by the National Institutes of Health (GM25422). We thank Professor R. F. Pasternack for valuable discussions and the use of the stopped-flow spectrometer and the Hewlett Packard Company for the loan of the 8450A rapid scan spectrophotometer.

Registry No. Catechol 1,2-dioxygenase, 9027-16-1; pyrogallol, 87-66-1; oxygen, 7782-44-7.

Enamidines. Versatile Vehicles for Homologation of Carbonyl Compounds

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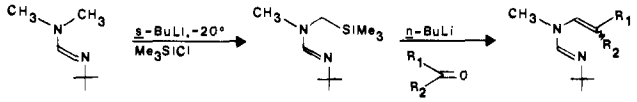
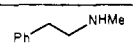
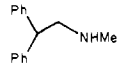
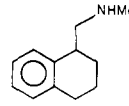
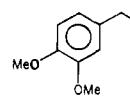
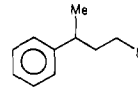
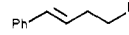
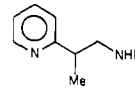
Enamidines **3** are rare in the literature, and their chemical behavior is virtually unexplored.[1] Yet, they possess a unique functional array since they may be considered as enamines containing an N-dipole stabilizing substituent,[2] i.e., formamidine. We report herein a simple route to enamidines but, more importantly, a preliminary study on their chemical properties which indicate that they indeed possess rich chemistry in areas of current synthetic interest, namely, homologation of carbonyl compounds.[3] The enamidines are readily prepared, in quantity, by metalation–silylation of **1**[4] to give the α-trimethylsilyl derivative **2**[5], which is metalated again and treated with various aldehydes or ketones in the Peterson olefination[6] to afford excellent yields of the enamidines **3**, as a mixture of geometric isomers. However, this



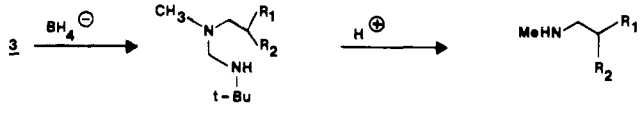
lack of stereoselectivity is of no consequence in the carbonyl homologations to follow. The carbonyl compounds employed to prepare **3** were transformed, from this versatile intermediate, to homologated amines (**5**), aldehydes (**6**), and ketones (**10**) by simple changes in procedure. The technique utilized[7] to prepare the

Table I. Homologation of Carbonyls to Amines **5** (Isolated Pure Material)

carbonyl	amine	% yield (from 2)	HCl salt[a] mp, °C
benzaldehyde		66	156–158
benzophenone		65	181–182
α-tetralone		66	202–205
veratraldehyde		67	138–140
α-(methylphenyl)-acetaldehyde		61	108–110
cinnamaldehyde		52	185–187[b]
α-acetylpyridine		70	*c*

[a] Mp of hydrochlorides agree with literature values where reported. [b] New compound; C, H, N analyses agree within ±0.4%. [c] Analyzed as free base.

N-methylamines involved addition of sodium borohydride in ethanol (−10 °C) under slightly acidic conditions (pH 6). This resulted in reduction of both the C=N link and the vinylamine moiety, producing the aminal **4** which was hydrolyzed with dilute acid to the amine **5**. It is also possible to carry out this entire



homologation from **1** without isolation of the intermediate silylformamidine **2** or purification of enamidine **3**[6] and aminal **4**. The intermediate silylformamidine, formed in situ, was immediately treated with *n*-butyllithium and the carbonyl compound to give enamidines **3** in 70–85%. Table I describes a number of examples which were examined. It is important to note that this procedure leads to *N*-methylamines as well as other *N*-alkylamines[8]

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(2) Beak, P.; Reitz, D. B. *Chem. Rev.* **1978**, 275. Krief, A. *Tetrahedron* **1980**, *36*, 2531.

(3) For a review on homologation of carbonyl compounds, see: Martin, S. F. *Synthesis* **1979**, 633.

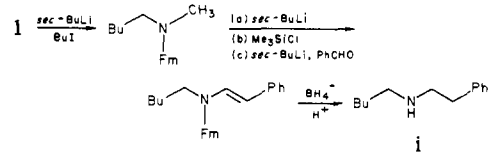
(4) Meyers, A. I.; Ten Hoeve, W. *J. Am. Chem. Soc.* **1980**, *102*, 7125.

(5) Preparation of **2**: *N,N*-dimethyl-*N'*-*tert*-butylformamidine (0.20 mol) in 400 mL of THF was treated with *sec*-butyllithium (0.22 mol) at −75 °C and the solution was allowed to warm to −20 °C over 30 min. After 1 h, the solution was recooled to −78 °C, and trimethylsilyl chloride (0.22 mol) was added and the mixture allowed to warm to the ambient temperature. The mixture was quenched in 600 mL of ice water and the organic layer removed by extraction with dichloromethane. Drying (Na_2SO_4), concentration, and distillation [bp 78–80° (7 mm)] gave 35.7 g of pure **2**; yield 89.2%; IR (neat) 1640 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.28 (s, 1 H), 2.80 (s, 3 H), 2.67 (s, 2 H), 1.12 (s, 9 H), 0.08 (s, 9 H).

(6) Peterson, D. J. *J. Org. Chem.* **1968**, *33*, 781. Preparation of **3** (R_1 = R_2 = Ph, typical procedure: A solution of 5 mmol of **2** in 10 mL of THF was cooled to −78 °C and treated with 5.75 mmol of *n*-butyllithium or *sec*-butyllithium and the solution allowed to warm to −20 ± 5 °C, stirred for 2 h, and recooled to −78 °C. A solution of benzophenone (5.75 mmol) in 4 mL of THF was added and the solution slowly allowed to warm to 0 °C. Quenching was performed in 20 mL of cold 10% bicarbonate and 40 mL of dichloromethane and the organic layer separated, washed (brine), dried (Na_2SO_4), and concentrated. The enamidines, thus obtained, may be used in the subsequent reactions described or may be purified by bulb-to-bulb distillation. For **3** (R_1 = R_2 = Ph) the distilled material, 5.41 g (93%), was recrystallized (pentane); mp 56–57 °C; IR (neat) 1642, 1614, 1592 cm^{-1}; ^1H NMR ($CDCl_3$) 7.42 (s, 1 H), 7.30 (s, 5 H), 7.25 (s, 5H), 6.57 (s, 1 H), 2.91 (s, 3 H), 1.03 (s, 9 H). Anal. ($C_{20}H_{24}N_2$) C, H, N.

(7) Procedure for conversion of **3** to amines **5**. The crude enamidine **3** (5 mmol) is dissolved in 15 mL of 80% ethanol and treated with 10% HCl until the pH of the solution is ~6. A solution of 600 mg of (15.9 mmol) sodium borohydride in 15 mL of ethanol is added dropwise between −5 and −15 °C, interrupted by dropwise addition of 10% HCl to maintain the pH at ~6. After stirring for 1 h at 0 °C, the mixture is made strongly alkaline (pH >12) by addition of NaOH pellets, diluted with 50 mL of water, extracted with ether, and then concentrated. The residue is redissolved in 30 mL of THF and treated with 5 mL of 10% HCl and the solution stirred at ambient temperature for 2 h. The solution is again made strongly alkaline (NaOH pellets), extracted with ether, dried (K_2CO_3), and then concentrated to provide the amine. Purification is accomplished by distillation or dry HCl (ether) to form the hydrochloride.

(8) Starting from **1**, it is possible to introduce, via metalation and alkylation, an alkyl group prior to metalation–silylation and then proceed to form *N*-alkylethylamines, i.e (Fm = formamidine),



This sequence was carried out without isolation or purification of any of the intermediates to give **i** in 55% overall yield.

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