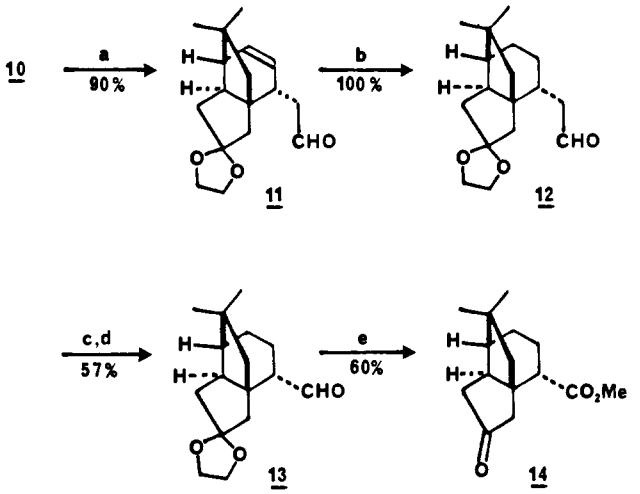
Scheme III[a]



[a] (a) Ethyl vinyl ether, $Hg(OAc)_2$; 250 °C, *o*-xylene, sealed tube. (b) H_2 (1 atm), 5% Pd/C, EtOH, 25 °C. (c) Ac_2O, KOAc, reflux. (d) OsO_4 (catalytic), $NaIO_4$ (8 equiv), 1:1 THF/H_2O; (e) Jones reagent; CH_2N_2, Et_2O.

Treatment of **10** with ethyl vinyl ether in the presence of mercuric acetate followed by heating the resultant allyl vinyl ether at 250 °C in a sealed tube in *o*-xylene afforded the γ,δ-unsaturated aldehyde **11** in 90% yield (56% conversion) (Scheme III).

With the critical C(8) stereocenter thus established, the formal total synthesis of (±)-quadrone followed directly. Catalytic hydrogenation [H_2 (1 atm), 5% Pd/C, EtOH] of **11** provided **12** in 100% yield. A one-carbon degradation of the aldehyde was then effected by enol acetylation (Ac_2O, KOAc, reflux)[13] followed by treatment with a catalytic amount of OsO_4 and $NaIO_4$ (8 eq) in 1:1 THF/H_2O at 25 °C.[14] The resultant aldehyde **13** was subjected to Jones oxidation and esterification (CH_2N_2, Et_2O, 0 °C → room temperature) to generate the keto ester **14** (mp 47–49 °C; lit.[2] 49–51 °C). Compound **14** proved to be identical by IR spectroscopy, MS, TLC, and 400-MHz ^1H NMR spectroscopy to a sample kindly provided by Professor Danishefsky.

Since **14** has been converted to (±)-quadrone (**1**),[2] the formal total synthesis was then complete. The transformation of the spiro[4.5]decadienone **2** to the keto ester **14** was accomplished in 21% overall yield. Further refinements of this scheme and efforts directed at a *de novo* lactone assembly (**12** → **1**) will be reported with full details in due course.[15]

Acknowledgment. Grateful acknowledgment is extended to the donors of the Petroleum Research Fund, administered by the American Chemical Society, the Research Corporation, the National Institutes of Health (Grant CA 25332), and the American Cancer Society (Grant IN-107F, awarded to C.W.M.) for their generous support of this research. High-field NMR spectra were obtained through the National Science Foundation Regional NMR Center at the University of South Carolina (Grant CHE 78-18723). We are especially grateful to Professor Samuel Danishefsky for a generous exchange of spectra and authentic samples.

Registry No. (±)-**1**, 74807-65-1; (±)-**2**, 80359-35-9; (±)-**4**, 80359-36-0; (±)-**5**, 80375-22-0; (±)-**6**, 80359-37-1; (±)-**7**, 80359-38-2; (±)-**8**, 80387-00-4; (±)-**9**, 80359-39-3; (±)-**10**, 80359-40-6; (±)-**11**, 80359-41-7; (±)-**12**, 80359-42-8; (±)-**13**, 80359-43-9; (±)-**14**, 78739-62-5.

(13) Bedoukian, P. A. "Organic Syntheses"; Wiley: New York, 1955; Coll. Vol. III, pp 127–129.
(14) Pappo, R.; Allen, D. S., Jr.; Lemieux, R. U.; Johnson, W. S. *J. Org. Chem.* **1956**, *21*, 478–479.
(15) The structural assignments of all compounds reported herein are fully supported by IR, MS, ^{13}C NMR, 400-MHz ^1H NMR data and elemental analysis.

Microsomal Reduction of the Carcinogen Chromate Produces Chromium(V)

K. Wetterhahn Jennette

Department of Chemistry, Dartmouth College Hanover, New Hampshire 03755

Received September 18, 1981

The metabolism of procarcinogens to reactive intermediates by cellular enzymes has been shown to be a critical step in the ability of many organic chemicals to react with nucleic acids and proteins in vivo and initiate cancer.[1] The importance of this process in carcinogenesis by inorganic chemicals has been unknown. We have previously reported that rat liver microsomes in the presence of NADPH enzymatically reduced chromate to chromium(III)[2] and that the electron-transport cytochrome P-450 system was responsible for the chromate-reductase activity of microsomes.[3] We now report that a stable reactive intermediate, chromium(V), is formed upon metabolism of the inorganic carcinogen chromate by rat liver microsomes in the presence of NADPH.

Incubation of chromate with rat liver microsomes and NADPH in 0.05 M Tris·HCl, pH 7.4, resulted in the appearance of an EPR signal with $g = 1.979$ and $\Delta H = 8.5$ G (Figure 1). No EPR signal was detected upon incubation of chromate with microsomes in the absence of NADPH. A 10- to 20-fold weaker signal was seen when chromate was incubated with NADPH in the absence of microsomes. The intensity of the signal increased with increasing concentration of microsomal protein. Peak intensity of the signal was optimized at a NADPH concentration of 0.85 mM by using a microsomal protein concentration of 11.6 mg/mL.

The EPR signal characteristic of chromium(V) appeared within 20 s after initiating the reaction of chromate with microsomes and NADPH at 22 °C. The intensity of the signal decreased rapidly in the first 2 min, increased slightly after 5 min, and then slowly decayed after 15 min (Figure 2). The signal persisted 80 min after initiating the reaction. The EPR signal observed 10 min after initiating the reaction was asymmetric (Figure 1) with a shoulder to lower field of the main peak. Power saturation studies revealed that this shoulder represented a distinct signal since it saturated at lower powers than the major $g = 1.979$ peak. This shoulder was absent in the 30-s sample. The appearance of the lower field signal at later times may account for the slight increase in peak intensity seen between 5 and 15 min (Figure 2).

Stable chromium(V) intermediates with $g \sim 1.98$ and $\Delta H =$ 2–20 G have been observed during the oxidation of organic acids, alcohols, and thiols.[4-9] EPR signals with $g \sim 1.98$ and $\Delta H =$ 7–17 G were detected in breast, liver, and thyroid tissues which had been incubated with solutions containing chromium(VI).[10] The present data indicate that the complete rat liver microsomal system is capable of producing two chromium(V) species upon metabolism of chromate. A direct one-electron transfer from the microsomal electron-transport cytochrome P-450 system to

(1) Miller, J. A.; Miller, E. C. In "Environmental Carcinogenesis"; P. Emmelot, E. Kriek, Eds.; Elsevier/North-Holland: Amsterdam, 1979; pp 25–50.
(2) Gruber, J. E.; Jennette, K. W. *Biochem. Biophys. Res. Commun.* **1978**, *82*, 700–706.
(3) Garcia, J. D.; Jennette, K. W. *J. Inorg. Biochem.* **1981**, *14*, 281–295.
(4) Kon, H. *J. Inorg. Nucl. Chem.* **1963**, *25*, 933–944.
(5) Garifyanov, N. S. *Dokl. Akad. Nauk SSSR* **1970**, *190*, 1368–1369.
(6) Galeev, A. M.; Usmanov, Z. I. *Zh. Strukt. Khim.* **1976**, *17*, 731–732.
(7) Mitewa, M.; Malinovski, A.; Bontchev, P. R.; Kabassanov, K. *Inorg. Chim. Acta* **1974**, *8*, 17–23.
(8) Mitewa, M. J.; Bontchev, P. R.; Kabassanov, K. S.; Petrov, G. N.; Russev, P. V.; Natzkova, M. D. *Dokl. Bolg. Akad. Nauk* **1978**, *31*, 1437–1440.
(9) Krumpolc, M.; DeBoer, B. G.; Roček, J. *J. Am. Chem. Soc.* **1978**, *100*, 145–153.
(10) Gutierrez, P. L.; Sarna, T.; Swartz, H. M. *Phys. Med. Biol.* **1976**, *21*, 949–954.

0002-7863/82/1504-0874$01.25/0

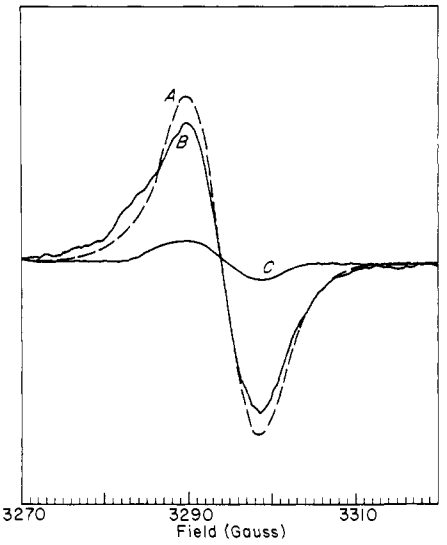


Figure 1. EPR spectra resulting from incubation of chromate (1.9 mM) with rat liver microsomes from phenobarbital-treated animals (11.6 mg/mL) and NADPH (0.88 mM) in 0.05 M Tris·HCl, pH 7.4 at 22 °C for (A) 0.5 min or (B) 10 min. (C) EPR spectrum resulting from incubation of chromate (1.9 mM) with NADPH (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).

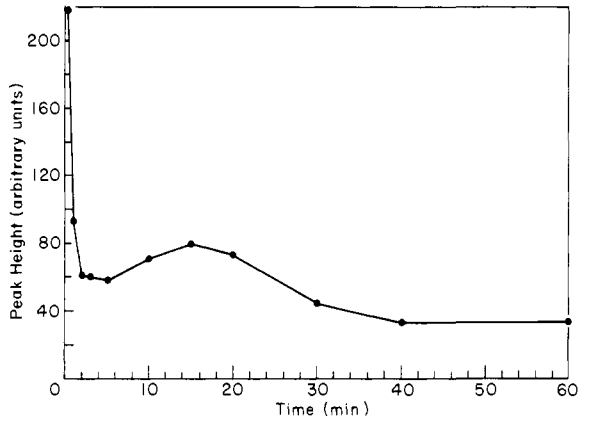


Figure 2. Time course for the appearance and loss of the EPR signal generated upon incubation of chromate, microsomes, and NADPH in 0.05 M Tris·HCl, pH 7.4, at 22 °C. Conditions were as described in Figure 1.

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.

Acknowledgment. This investigation was supported by Grant CA 23108, awarded by the National Cancer Institute, DHEW, and an A. P. Sloan Research Fellowship. I thank J. Warden, Rensselaer Polytechnic Institute, for his generous help in performing the EPR measurements, and I thank J. Peisach and J. Freedman, Albert Einstein College of Medicine, for the initial studies which demonstrated the feasibility of this project.

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

(11) Beattie, J. K.; Haight, G. P. *Prog. Inorg. Chem.* **1972**, *17*, 93-145.

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

Lawrence Que, Jr.,* and Ruth Mayer†

Department of Chemistry, Baker Laboratory Cornell University, Ithaca, New York 14853

Received September 28, 1981

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.

(1) Entsch, B.; Ballou, D. P.; Massey, V. *J. Biol. Chem.* **1976**, *251*, 2550–2563. Entsch, B.; Ballou, D. P.; Husain, M.; Massey, V. *Ibid.* **1976**, *251*, 7367–7379. Entsch, B.; Husain, M.; Ballou, D. P.; Massey, V.; Walsh, C. *Ibid.* **1980**, *255*, 1420–1429.

(2) Gunsalus, I. C.; Meeks, J. R.; Lipscomb, J. D.; Debrunner, P.; Munck, E. In "Molecular Mechanisms of Oxygen Activation"; Hayaishi, O., Ed.; Academic Press: New York, 1974; Chapter 14.

(3) Ishimura, Y.; Nozaki, M.; Hayaishi, O.; Nakamura, T.; Tamura, M.; Yamazaki, I. *J. Biol. Chem.* **1970**, *245*, 3593–3602. Hirata, F.; Ohnishi, T.; Hayaishi, O. *Ibid.* **1977**, *252*, 4637–4642.

(4) Que, L., Jr. *Struct. Bonding (Berlin)* **1980**, *40*, 39–72.

(5) Fujisawa, H.; Hiromi, K.; Uyeda, M.; Nozaki, M.; Hayaishi, O. *J. Biol. Chem.* **1971**, *246*, 2320–2321.

(6) Fujisawa, H.; Hiromi, K.; Uyeda, M.; Okuno, S.; Nozaki, M.; Hayaishi, O. *J. Biol. Chem.* **1972**, *247*, 4422–4428.

(7) Ballou, D. P.; Bull, C. In "Biochemical and Clinical Aspects of Oxygen"; Caughey, W., Ed.; Academic Press: New York, 1978; pp 573–585.

(8) Bull, C.; Ballou, D. P.; Otsuka, S. *J. Biol. Chem.* **1981**, *256*, 12681–12686.

(9) Que, L., Jr.; Epstein, R. M. *Biochemistry* **1981**, *20*, 2545–2549.

(10) Tatsuno, Y.; Saeki, Y.; Iwaki, M.; Yagi, T.; Nozaki, M.; Kitagawa, T.; Otsuka, S. *J. Am. Chem. Soc.* **1978**, *100*, 4614–4615. Keyes, W. E.; Loehr, T. M.; Taylor, M. L. *Biochem. Biophys. Res. Commun.* **1978**, *83*, 941–945. Felton, R. H.; Cheung, L. D.; Phillips, R. S.; May, S. W. *Ibid.* 1978, *85*, 844–850. Bull, C.; Ballou, D. P.; Salmeen, I. *Ibid.* **1979**, *87*, 836–841.

(11) Que, L., Jr.; Heistand, R. H., II *J. Am. Chem. Soc.* **1979**, *101*, 2219–2221. Que, L., Jr.; Heistand, R. H., II; Mayer, R.; Roe, A. L. *Biochemistry* **1980**, *19*, 2588–2593.

(12) Keyes, W. E.; Loehr, T. M.; Taylor, M. L.; Loehr, J. S. *Biochem. Biophys. Res. Commun.* **1979**, *89*, 420–427.

(13) Nakata, H.; Yamauchi, T.; Fujisawa, H. *Biochem. Biophys. Acta* **1978**, *527*, 171–181.

(14) May, S. W.; Phillips, R. S. *Biochemistry* **1979**, *18*, 5933–5939.

(15) The enzyme was purified from *Pseudomonas arvilla* C-1 according to the procedure of Fujiwara, et al. Fujiwara, M.; Golovleva, L. A.; Saeki, Y.; Nozaki, M.; Hayaishi, O. *J. Biol. Chem.* **1975**, *250*, 4848–4855.

(16) Saeki, Y.; Nozaki, M.; Senoh, S. *J. Biol. Chem.* **1980**, *255*, 8465–8471.

0002-7863/82/1504-0875$01.25/0