Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 6, No. 11, pp. 1285-1288, 1996

0960-894X/96 $15.00 + 0.00

PII: S0960-894X(96)00217-X

AN ELECTROCHEMICAL STUDY TO MODEL THE CHORISMATE SYNTHASE REACTION

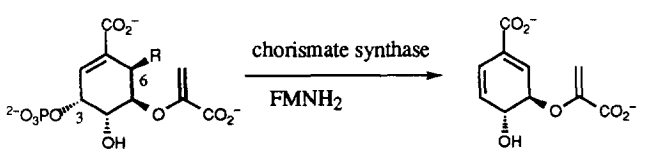
Maria-Elena Theoclitou, Peter J. Duggan@ and Chris Abell*

University Chemical Laboratory, Lensfield Road, Cambridge CB2 1EW, UK
FAX: 44 (0)1223 336362, e-mail: ca26@cus.cam.ac.uk

Abstract The electrochemical reduction of 3-phosphate-cyclohex-1-ene-1-carboxylic acid **13** has been studied as a model for the chorismate synthase reaction. An electrochemical reaction occurs only when **13** is fully protonated. However the reaction results in loss of the carboxyl group rather than the phosphate.

Chorismate synthase is the last enzyme on the trunk of the shikimate pathway before it branches at chorismate to form the aromatic amino acids and other aromatic compounds.[1] It catalyses the conversion of enol pyruvoyl shikimate 3-phosphate (EPSP) **1** to chorismate **2** (Scheme 1). The reaction involves the loss of the C-3 phosphate group and removal of the hydrogen from the 6-pro*R* position.[2] Although the reaction can be considered formally as an E2' elimination, the mechanism is not known in any detail. The importance of this reaction, and the general paucity of mechanistic information about reactions involving cleavage of bonds from carbon to a phosphate group, has prompted us to study both the enzyme reaction and the non-enzyme reaction.

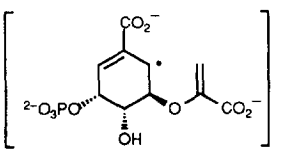
Scheme 1



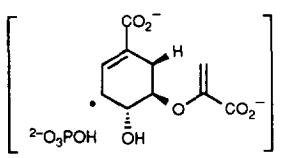
1 R= H

3 R = F

2



4



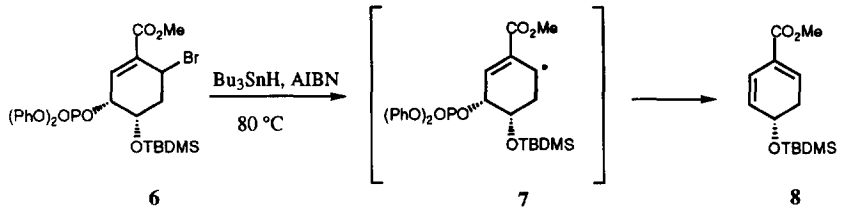
5

The enzyme mechanism has recently been the focus of intense study by several groups. We have shown that the reaction proceeds with a kinetic isotope effect due to deuteriation at C-6,[3] and that the rehybridisation at C-3 is partially rate limiting.[4] The enzyme reaction has an absolute requirement for a reduced flavin cofactor ($FMNH_2$),[5] and there are spectroscopic changes in the flavin associated with catalysis.[6] These observations prompted speculation that the reaction may involve radical intermediates. Subsequently it was shown that incubation of chorismate synthase with *(6R)*-6-fluoroEPSP **3** leads to irreversible formation of the semiquinone radical form of the flavin on the enzyme.[7] If the reaction with EPSP does go via a one electron mechanism, two different substrate radical intermediates can be envisaged (Scheme 1), depending on whether a hydrogen atom is

@ Now at: Dept of Molecular Sciences, James Cook University, Townsville, Queensland 4811, Australia.

abstracted from C-6 to form **4**, or whether electron donation to EPSP triggers cleavage of the C-3 phosphate (C-OP) bond to give **5**.

Scheme 2

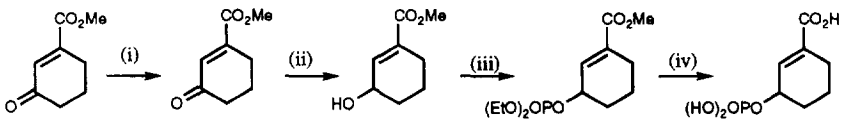


6 **7** **8**

In a recent model study Giese generated **7** as a model for **4** (Scheme 2), and isolated 40% of the (chorismate-like) elimination product **8**.[8] In this paper we attempt to generate a radical equivalent to **5** by electrochemical reduction of model compounds **12** and **13**. The reduction could occur by putting an electron either into the π system of the α,β–unsaturated carboxyl group[9] or onto the phosphate, to promote C-OP bond cleavage. There is good precedent for the reductive loss of a leaving group (acetate) adjacent to an α,β–unsaturated carboxyl in the electrochemical reduction of cephalosporanic acids.[10] There is also precedent for loss of phosphate adjacent to a radical centre in studies on DNA cleavage.[11]

Our first synthetic target was the fully protected phosphotriester **12**. The synthesis from methyl cyclohexene-1-carboxylate **9** is shown in Scheme 3. Allylic oxidation using chromium trioxide in acetic acid-water forms the 3-keto compound **10**, which is then reduced to the allylic alcohol **11** by sodium borohydride. The phosphotriester **12** was formed by reacting **11** with diethyl phosphochloridate in the presence of 1-methylimidazole in anhydrous ether. The fully-deprotected carboxylic acid phosphate **13** was then obtained by sequential base and acid hydrolyses.[12]

Scheme 3



9 **10** **11** **12** **13**

(i) CrO_3, AcOH, H_2O, 2h at 20°C then 16 h at 80°C, 34% (ii) $NaBH_4$, MeOH, 23°C, 46 h, 65% (iii) $(EtO)_2POCl$, 1-methylimidazole, Et_2O, 0°C, 1.5 h, 70% (iv) (a) K_2CO_3, MeOH, 23°C, 24h, 61% (b) HCl, 23°C, 1 h, 90%

The electrochemical experiments were carried out under argon in an electrochemical cell with a platinum working electrode, and tetra-*n*-butylammonium perchlorate in dimethyl formamide as the electrolyte.[13] Cyclic voltammograms were recorded by scanning from 0 V to -2.8 V at a standard rate of 100 mV sec^{-1}. The cyclic voltammogram showed no signal above the background signal when the fully protected triester **12** (2 mM) was dissolved in the electrolyte. The triester was therefore hydrolysed to the carboxylate phosphate **13** to more closely model the substrate in the enzymatic reaction. Solutions of **13** were adjusted to pH 1.5, 2.3, 6.0, and 9.5 using sodium hydroxide before being lyophilised to generate **13** predominantly as the triacid, and the mono-, di and tri-sodium salts respectively. Each of these different species was used in electrochemical experiments.

Figure 1 shows the cyclic voltammogram obtained for **13** (sample pH previously adjusted to 1.5). There is a concentration-dependent signal at *ca.* -2.1 V corresponding to a one electron reaction. The absence of a reverse peak suggests the process is either irreversible or the electrochemical step is followed by a fast chemical step, i.e. an electrochemical reaction. Diagnostic tests confirmed an electrochemical reaction was occuring:

$I_p/v^{1/2}$ decreased slightly with increasing v (I_p is the current and v the scan rate); and the peak potential became marginally more negative with increasing scan rate. This latter effect was more marked in the presence of FMN (0.4 mM), as would be expected if FMN reduced the size of the transfer coefficient. A weaker signal was seen in the cyclic voltammogram recorded for the sample of **13** prepared at pH 2.3, but no signals were seen for the more alkaline samples. This suggests that the electrochemical reaction requires **13** to be fully protonated.

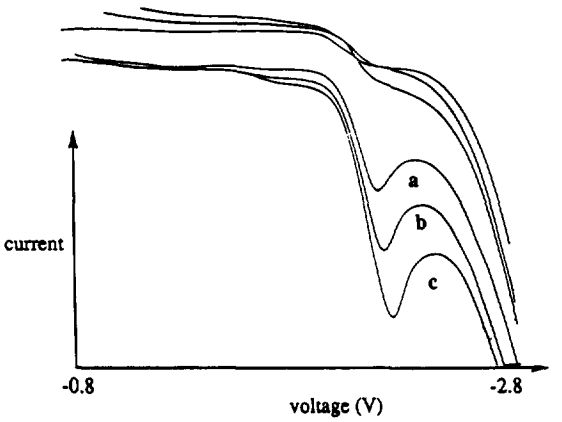
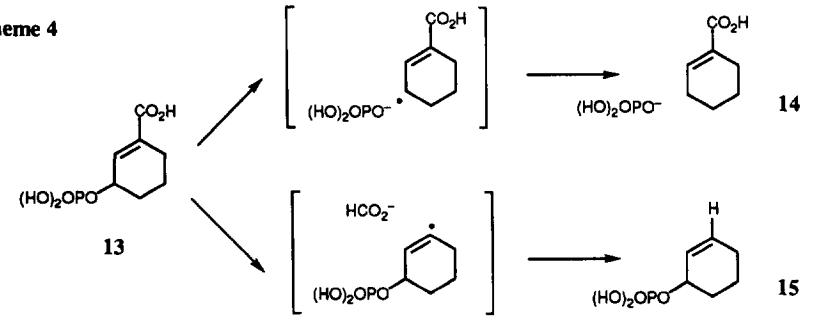


Figure 1 Cyclic voltammogram of a solution of **13** (2 mM) scanned from -0.8 V to -2.8 V. The scan rates are (a) 50, (b) 100, and (c) 200 mV s^{-1}. The background signal is from reduction of the tetraalkyl ammonium cation.

In order to identify the product of the electrochemical reaction, **13** (12 mM) was electrochemically reduced at a constant voltage of -2.1 V. A product formed slowly (11% conversion after 16 h). This was isolated and purified by preparative thin layer chromatography. The structure was unambiguously assigned as cyclohex-2-enyl phosphate **15**, formed by decarboxylation of **13**.[14] There was no evidence for **14**, the product expected from reductive cleavage of the C-OP bond. The cleavage could either generate the vinyl radical (as shown) or the vinyl anion as an intermediate. The observed decarboxylation is reminiscent of the electrochemical *oxidation* of carboxylate anions (the Kolbe reaction).

Scheme 4



The experiment described in this paper shows that a model system with a phosphate next to an α,β-unsaturated acid does not undergo C-OP cleavage under strongly reducing electrochemical conditions. In the

context of the chorismate synthase reaction, this result contrasts with the observation of C-OP bond cleavage to form a chorismate-like diene when the C-6 radical is generated from **6**.[8] However whereas it is possible to envisage electron donation to EPSP **1** from the enzyme-bound reduced flavin to form radical **5**, it is difficult to see how the radical **4** could be formed on the enzyme. Although the electrochemical experiments described were intended to model the chorismate synthase reaction, the observed decarboxylation has similarities to the reaction catalysed by pyruvate formate-lyase. This enzyme uses a radical process to cleave the C1-C2 bond of pyruvate and release the carboxyl group as formate.[15]

Acknowledgement We gratefully acknowledge the financial support of the BBSRC for a postdoctoral award to PD and M-E T. We thank Adham Ramzi Ramadan, Dr Justin Gooding, Dr Carl Hall, Dr Lisa Hall, and Dr Trevor Rayment for helpful advice.

References

1 Bentley, R. *Crit. Rev. in Biochem. and Mol. Biol.* **1990**, *25*, 307-384.
2 Onderka, D. K.; Floss, H. G. *J. Am. Chem. Soc.* **1969**, *91*, 5894-5896.
3 Balasubramanian, S.; Abell, C.; Coggins, J. R. *J. Am. Chem. Soc.* **1990**, *112*, 8581-8583.
4 Balasubramanian, S.; Coggins, J. R.; Abell, C. *Biochemistry* **1995**, *34*, 341-348.
5 Ramjee, M. K.; Coggins, J. R.; Hawkes, T. R.; Lowe, D. J.; Thorneley, R. N. F. *Bioorg. Med. Chem. Lett.* **1993**, *3*, 1409-1414.
6 Ramjee, M. N.; Coggins, J. R.; Hawkes, T. R.; Lowe, D. J.; Thorneley, R. N. F. *J. Am. Chem. Soc.* **1991**, *113*, 8566-8567.
7 Ramjee, M. N.; Balasubramanian, S.; Abell, C.; Coggins, J. R.; Davies, G. M.; Hawkes, T. R.; Lowe, D. J.; Thorneley, R. N. F. *J. Am. Chem. Soc.* **1992**, *114*, 3151-3153.
8 Giese, B.; Almstead, N. G. *Tetrahedron Lett.* **1994**, *35*, 1677-1680.
9 The half-wave potential for ethyl acrylate is -2.12 V; Baizer, M. M.; Chruma, J. L. *J. Org. Chem.* **1972**, *37*, 1951-1960.
10 Ochiai, M.; Aki, O.; Morimoto, A.; Okada, T.; Shinozaki, K.; Asahi, Y. *J. Chem. Soc. Perkin Trans I* **1974**, 258-262.
11 Giese, B.; Erdmann, P.; Giraud, L.; Gobel, T.; Petretta, M.; Schafer, T. *Tetrahedron Lett.* **1994**, *35*, 2683-2686.
12 Methyl 3-diethoxyphosphoryloxycyclohex-1-ene-1-carboxylate **12**: R_f 0.50 (hexane / ethyl acetate 1:1); δ_H (250 MHz, $CDCl_3$) 6.85 (1H, bd, J = 1.9 Hz, H-2), 4.95 (1H, br s, H-3), 4.05 (4H, qn, J = 7.5 Hz, CH_2 of Et), 3.75 (3H, s, CO_2Me), 2.30-2.00 (2H, m, H-6), 1.96-1.90 (1H, m), 1.86-1.70 (2H, m), 1.68-1.5 (1H, m), 1.30 (6H, t, J = 6.0 Hz, CH_3 of Et); ν_{max} (liq.film) 1740 (ester C=O), 1640 (C=C), 1250 (P=O); m/z 293 (M^+), 278 ([M - Me]$^+$), 261 ([M - MeOH]$^+$), 234 ([M - CO_2Me]$^+$). 3-Dihydroxyphosphoryloxycyclohex-1-ene-1-carboxylic acid **13**: δ_H (250 MHz, $CDCl_3$) 10.56 (1H, s, COOH), 6.90 (1H, bd, H-2), 4.82 (1H, br s, H-3), 2.3-2.05 (2H, m, H-6), 1.94-1.80 (1H, m), 1.78-1.6 (2H, m), 1.57-1.40 (1H, m); δ_P (101 MHz, D_2O, pD =7.0, ref. to TMP) -140.2; ν_{max} (liq.film) 3300 (OH), 2750 (P-OH), 1720 (C=O), 1640 (C=C), 1250 (P=O); m/z 223 (M^+).
13 The electrochemical cell was constructed of a short, thick-walled test tube capped with a plug with holes to allow the insertion of the electrodes into the solution. It contained a platinum wire working electrode, a platinum button counter electrode and a silver / silver chloride reference electrode. The electrolyte was *n*-Bu_4NClO_4 (0.51 g, 1.5 mmoles) in anhydrous dimethyl formamide (15 ml). The solution was deoxygenated with a stream of argon passing through the solution for 30 min. The stream was then removed from the solution, but left in the cell so that the actual entire experiment was carried out under a blanket of argon. A cyclic voltammogram was recorded. Scanning was from 0 V to -2. 8 V versus the reference electrode, at a standard scan rate of 100 mV s^{-1}. When a reproducible background signal had been obtained, the substrate was added to produce a 2 mM solution in dimethyl formamide. The solution was deoxygenated for a further 10 min and a cyclic voltammogram run.
14 Data for **15** δ_H (500 MHz, CDCl3) 5.87 (1H, d, H-1(CH=C)), 5.80 (1H, d, H-2 (CH=C), 3.61 (1H, dt, H-3), 2.05-2.00 (2H, m, H-6), 1.86-1.82 (2H, m, H-4) and 1.58-1.51 (2H, qn, J= 5.0 Hz, H-5); ν_{max} (liq.film) 2820 (P-OH), 1650 (C=C), 1260 (P=O); m/z (FAB, glycerol) 178 (M^+).
15 Wagner, A. F. V.; Frey, M.; Neugebauer, F. A.; Schafer, W.; Knappe, J. *Proc. Natl. Acad. Sci. U.S.A.* **1992**, *89*, 996-1000.

(*Received in Belgium* 28 *March* 1996; *accepted* 7 *May* 1996)