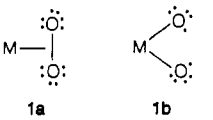
of the O_{1s} peaks at 530.1 and 528.4 eV are consistent with a formulation of $Ba^{2+}Pb^{2+}[O_2]^{2-}O^{2-}$. The O_{1s} binding energy for peroxide dianions in Na_2O_2 and BaO_2 are 530.4 and 530.9 eV, respectively; O^{2-} binding energies are about 528-529 eV.[9] The absence of a feature at about 289 eV rules out the presence of CO_3^{2-} on the surface whose O_{1s} spectrum interferes with that of peroxide dianion.[10] The Pb $4f_{7/2}$ photoelectron peak was markedly asymmetric; qualitatively similar XPS peak shapes have been observed in both atomic and synthetic metals.[11] The surface composition is $Ba_{1.0}Pb_{1.7}[O_2^{2-}]_{2.0}O^{2-}_{2.1}$.

The photoelectron spectrum, shown in the upper traces of Figure 1, of a used catalyst after several hundred turnovers is more complex than that of the unused catalyst. New features at 534.1 and 292.5 eV in the O_{1s} and C_{1s} regions, respectively, are assigned to the HCO_3^- ion since the O/C atomic intensity ratio is 3.1 and the binding energies for these two new peaks are similar to those observed in $NaHCO_3$.[9] A broad, unresolved signal extending from 285 to 289.5 eV appears different from the high binding energy shoulder of adventitious carbon in the O_2-annealed sample and probably includes some CO_3^{2-}. If the 531.3 eV O_{1s} peak were derived solely from carbonate, the corresponding C_{1s} carbonate peak would be both very broad (>3 eV) and centered at unreasonably low binding energies (<288.5 eV). Thus the 531.3 eV feature arises from both carbonate and the peroxide of $BaPbO_3$. Therefore the 528.2 O_{1s} peak and the Pb $4f_{7/2}$ peak at 137.4 eV (not shown) are assigned to $BaPbO_3$. The Ba $3d_{5/2}$ spectrum (not shown) may be deconvoluted into three Gaussian components that correspond to $BaPbO_3$, $Ba(HCO_3)_2$, and $BaCO_3$. The overall surface composition is $Ba_{3.9}Pb_{1.0}C_{2.7}O_{8.7}$. These results indicate that (1) $BaPbO_3$, hence the peroxide dianion, remains at the surface, (2) the surface becomes partially covered with $BaCO_3$ and $Ba(HCO_3)_2$, and (3) the surface becomes deficient in lead. Bulk analytical results show that about 20% of the lead is lost under relevant catalytic conditions.

The unusual nature of the peroxide ions in $BaPbO_3$ deserves comment. Relativistic stabilization of the Pb 6s band[12] may cause an oxidation of the O 2p band with the result that $BaPbO_3$ is better described as $Ba^{2+}Pb^{2+}[O_2]^{2-}O^{2-}$ rather than $Ba^{2+}Pb^{4+}[O^{2-}]_3$. Independent evidence,[13] consistent with this hypothesis, is provided by an analysis of the IR reflectance spectra of the $BaPb_{1-x}Bi_xO_3$ system which indicates unusually low effective charges on the oxygen atoms that vary from -1.18 for $x = 1$ to -0.6 for $x = 0.35$. The XPS and IR results[13] provide evidence concerning charge distribution, not local geometry. *Classical* inorganic peroxides exist as diatomic, through-space spin-coupled species **1a** with O-O distances of 1.45-1.50 Å.[14] Crystallographic analyses[15] of $BaPbO_3$, based on a profile refinement of powder data and which unfortunately includes high-R factors, indicates O···O contacts of ~3.0 Å. These results suggest that a *nonclassical* through-bond species **1b** may occur in $BaPbO_3$ and perhaps in other superconducting oxides[8,16] reported to exhibit similar XPS features attributed to peroxide anions, as well.



1a **1b**

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Registry No. $BaPbO_3$, 12047-25-5; CH_4, 74-82-8; C_2H_4, 74-85-1; C_2H_6, 74-84-0; Na_2O_2, 1313-60-6; BaO_2, 1304-29-6; O^{2-}, 16833-27-5.

(9) Kharas, K. C. C., unpublished results.
(10) (a) Prabhakaran, K.; Sen, P.; Rao, C. N. R. *Surf. Sci.* **1986**, *177*, L971-L977. (b) Prince, K. C.; Paolucci, G. *J. Elec. Spectrosc. Relat. Phenom.* **1985**, *37*, 181-185.
(11) (a) Wertheim, G. K.; Citrin, P. H. *Topics Appl. Phys.* **1978**, *26*, 197-236. (b) Chiang, S.; Wertheim, G. K.; DiSalvo, F. J. *Solid State Chem.* **1976**, *19*, 75-78.
(12) (a) Pitzer, K. S. *Acc. Chem. Res.* **1979**, *12*, 270-276. (b) Pyykkö, P.; Desclaux, J.-P. *Acc. Chem. Res.* **1976**, *12*, 276-281. (c) Pyykkö, P. *Adv. Quantum Chem.* **1978**, *11*, 353-409. (d) Christiansen, P. A.; Ermler, W. C.; Pitzer, K. S. *Ann. Rev. Phys. Chem.* **1985**, *36*, 407-432.
(13) Uchida, S.; Tajima, S.; Masaki, A.; Sugai, S.; Kitazawa, K.; Tanaka, S. *J. Phys. Soc. Jpn.* **1985**, *54*, 4395-4409.
(14) (a) Vannerberg, N.-G. *Prog. Inorg. Chem.* **1962**, *4*, 125-197. (b) Boca, R. *Coord. Chem. Rev.* **1983**, *50*, 1-72.
(15) (a) Cox, D. E.; Sleight, A. W. *Proc. Conf. Neutron Scattering* **1976**, 45-54. (b) Thornton, G.; Jacobson, A. J. *Mat. Res. Bull.* **1976**, *11*, 837-842.
(16) (a) Dai, Y.; Manthiram, A.; Campion, A.; Goodenough, J. B. *Phys. Rev. B* **1988**, *38*, 5091-5094. (b) Steiner, P.; Hüfner, S.; Kinsinger, V.; Sander, I.; Siegwart, B.; Schulz, R.; Junk, S.; Schwitzgebel, G.; Gold, A.; Politis, C.; Müller, H. P.; Hoppe, R.; Kemmler-Sack, S.; Kunz, C. *Z. Phys. B* **1988**, *69*, 449-458.
(17) Suib, S. L.; Winiecki, A. M.; Kostapapas, A. *Langmuir* **1987**, *3*, 483-488.

Potent Inhibition of 5-Enolpyruvylshikimate-3-phosphate Synthase by a Reaction Intermediate Analogue

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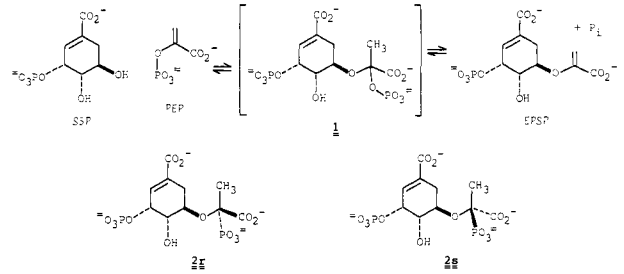
5-Enolpyruvylshikimate-3-phosphate synthase (EPSP synthase, E.C. 2.5.1.19) catalyzes the reversible transfer of the carboxyvinyl moiety of phosphoenolpyruvate (PEP) to the 5-hydroxyl group of shikimate 3-phosphate (S3P). From a commercial standpoint, it is the most important enzyme in the shikimic acid pathway since it is the target of the broad spectrum herbicide glyphosate (*N*-phosphonomethylglycine).[1-3] A number of isotope labeling and kinetic studies[4,5] have supported the proposal of Levin and Sprinson[6] that the enzymatic reaction proceeds via an addition-elimination mechanism (Scheme I), and the isolation of tetrahedral adduct **1** by Anderson et al.[7] has provided the final proof. We now report the synthesis of the two diastereomers of phosphonate **2**, a stable analogue of intermediate **1**, and their evaluation as reaction intermediate analogue inhibitors of EPSP synthase.

Inhibitors **2r** and **2s** are synthesized from (-)-shikimic acid as shown in Scheme II. Rhodium diacetate-catalyzed coupling[8] of acetonide **3**[9] with methyl (dibenzylphosphono)diazoacetate **4**,[10] followed by alkylation of the potassium salt of the resulting ether **5**, affords compound **6** in 63% overall yield. Acid-catalyzed cleavage of the acetonide moiety, followed by cyclization to lactone **7**, takes place in 79% yield. The free hydroxyl group of **7** is

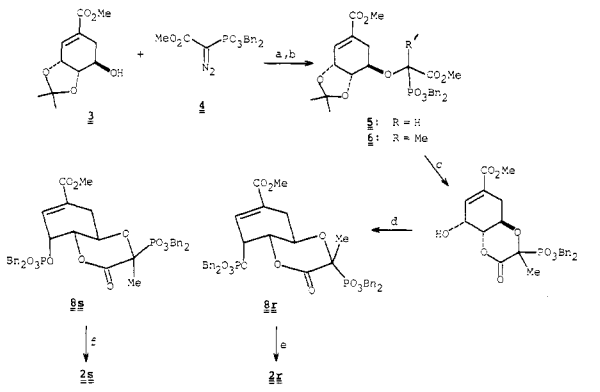
(1) Steinrücken, H. C.; Amrheim, N. *Biochem. Biophys. Res. Commun.* **1980**, *94*, 1207-1212.
(2) Boocock, M. R.; Coggins, J. R. *FEBS Lett.* **1983**, *154*, 127-133.
(3) Anderson, K. S.; Sikorski, J. A.; Johnson, K. A. *Biochemistry* **1988**, *27*, 1604-1610.
(4) Bondinell, W. E.; Vnek, J.; Knowles, P. F.; Sprecher, M.; Sprinson, D. B. *J. Biol. Chem.* **1971**, *246*, 6191-6196. Ife, R. J.; Ball, L. F.; Lowe, P.; Haslam, E. *J. Chem. Soc., Perkin Trans. 1.* **1976**, 1776-1783. Grimshaw, C. E.; Sogo, S. G.; Knowles, J. R. *J. Biol. Chem.* **1982**, *257*, 596-598. Anton, D. L.; Hedstrom, L.; Fish, S. F.; Abeles, R. H. *Biochemistry* **1983**, *22*, 5903-5908. Grimshaw, C. E.; Sogo, S. G.; Copley, S. D.; Knowles, J. R. *J. Am. Chem. Soc.* **1984**, *106*, 2699-2700. Asano, Y.; Lee, J. J.; Shieh, T. L.; Spreafico, F.; Kowal, C.; Floss, H. G. *J. Am. Chem. Soc.* **1985**, *107*, 4314-4320. Wibbenmeyer, J.; Brundage, L.; Padgette, S. R.; Likos, J. J.; Kishore, G. *Biochem. Biophys. Res. Commun.* **1988**, *153*, 760-766.
(5) Anderson, K. S.; Sikorski, J. A.; Johnson, K. A. *Biochemistry* **1988**, *27*, 7395-7406.
(6) Levin, J. G.; Sprinson, D. B. *J. Biol. Chem.* **1964**, *239*, 1142-1150.
(7) Anderson, K. S.; Sikorski, J. A.; Benesi, A. J.; Johnson, K. A. *J. Am. Chem. Soc.* **1988**, *110*, 6577-6579.
(8) Ganem, B.; Ikota, N.; Muralidharan, V. B.; Wade, W. S.; Young, S. D.; Yukimoto, Y. *J. Am. Chem. Soc.* **1982**, *104*, 6787-6788. Paquet, F.; Sinay, P. *Tetrahedron Lett.* **1984**, *25*, 3071-3074.
(9) Fischer, H. O. L.; Dangschat, G. *Helv. Chim. Acta* **1935**, *18*, 1206-1213.
(10) Prepared from the phosphonoacetate in analogy to the following: Regitz, M.; Anschütz, W.; Liedhegener, A. *Chem. Ber.* **1968**, *101*, 3734-3743.

0002-7863/89/1511-2337$01.50/0

Scheme I



Scheme II[a]



[a] (a) $Rh_2(OAc)_4$, benzene, Δ; (b) KH, MeI, THF (63%); (c) *p*-TsOH, aqueous MeCN; *p*-TsOH, benzene, Δ (79%); (d) LDA, $Bn_4P_2O_7$, THF, −78 → 10 °C (25% → **8r**, 46% → **8s**); (e) TMSiBr; aqueous NaOH (45%); (f) TMSiBr; aqueous NaOH (81%).

Table I. Inhibition of EPSP Synthase[a]

inhibitor	competing substrate[b]	K_i (μM)
2r	EPSP	0.015 ± 0.001
2r	P_i	0.09 ± 0.01
2s	EPSP	1.13 ± 0.07
2s	P_i	2.1 ● 0.2
glyphosate	(apparent K_I[c])	0.4

[a] Determined at 25 °C in potassium–HEPES buffer, pH 7.5. [b] For experiments in competition with EPSP, $[P_i]$ = 50 mM (K_m = 1.36 ± 0.06 mM); for experiments in competition with P_i, [EPSP] = 50 μM (K_m = 2.32 ± 0.12 μM). [c] Glyphosate binds to the E·S3P complex (Padgette et al., ref 16).

phosphorylated by formation of the lithium alkoxide and treatment with tetrabenzyl pyrophosphate[11,12] to provide the diastereomers **8r** and **8s** in 25% and 46% yield, respectively, after chromatographic separation. The stereochemistry of these isomers was assigned by 2D NMR.[13] Deprotection is accomplished by treatment with trimethylsilyl bromide (to cleave the phosphorus benzyl esters[15]) and aqueous base (to hydrolyze the methyl ester and lactone moieties), and the inhibitors are isolated as their sodium salts in good overall yield after purification by ion exchange chromatography (**2r**, 45% yield; **2s**, 81% yield).

Analogues **2r** and **2s** were evaluated as inhibitors of EPSP synthase from *Petunia hybrida*.[16] The enzymatic reaction was

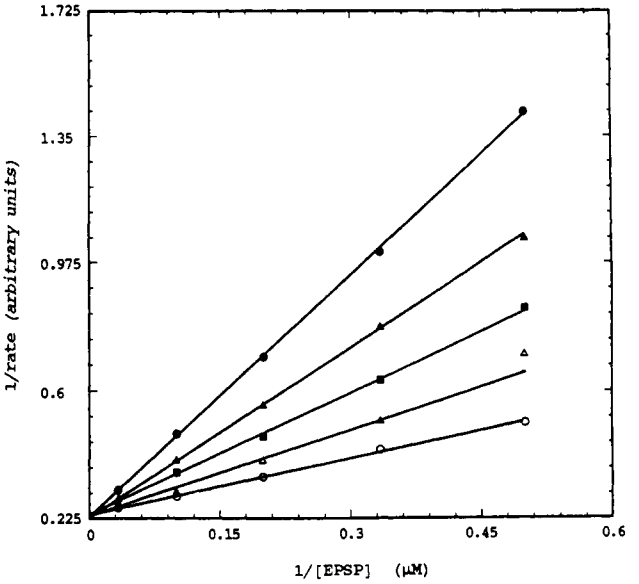


Figure 1. Lineweaver–Burk plot for inhibition of EPSP synthase by isomer **2r** in competition with EPSP: $[P_i]$ = 50 mM, [**2r**] = 0 (○), 6 nM (△), 10 nM (■), 25 nM (▲), and 50 nM (●). Each point represents an average of two or three determinations; the data were fit using Cleland's COMP program.[19]

followed in the reverse direction (EPSP + P_i → PEP + S3P), using a coupled assay with pyruvate kinase and lactate dehydrogenase to allow PEP release to be monitored spectrophotometrically.[2,17] The inhibitors were evaluated both by varying EPSP at saturating concentrations of phosphate (50 mM) and by varying phosphate at saturating EPSP (50 μM) (Table I, Figure 1). The kinetic behavior of these inhibitors reveals that they are competitive with respect to both EPSP and phosphate. In the ordered binding sequence described by Anderson et al.,[5] EPSP binds first, so that competition with this substrate is to be expected. Because substrate equilibration is rapid and phosphate can pull the equilibrium, E + EPSP → E·EPSP by converting E·EPSP to E·EPSP·P_i, inhibition can also be overcome at high phosphate concentrations, even though this substrate and the inhibitors do not compete for the same form of the enzyme.[18]

With a K_i of 15 nM, isomer **2r** represents the most potent inhibitor of EPSP synthase yet reported, binding more than an order of magnitude more tightly than glyphosate. It thus conforms to the expectation that an enzyme should show high affinity for a mimic of an unstable reaction intermediate as well as to transition state analogues. Also important is the fact that the *R*-inhibitor binds two orders of magnitude more tightly than the *S*-diastereomer **2s**. If, as seems likely, the phosphonate group of these inhibitors prefers to bind in the site normally occupied by the phosphate moiety of **1**, we can infer that the side chain of intermediate **1** also has the *R* configuration.

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Supplementary Material Available: Full experimental procedures and characterization for all compounds and enzyme assays described (10 pages). Ordering information is given on any current masthead page.

(11) Khorana, H. G.; Todd, A. R. *J. Chem. Soc.* **1953**, 2257–2260.
(12) Chouinard, P. M.; Bartlett, P. A. *J. Org. Chem.* **1986**, *51*, 75–78.
(13) In the 2D ROESY spectrum[14] of **8s**, but not that of **8r**, a nuclear Overhauser effect is observed between the side chain methyl group and the hydrogen at C-5 (shikimate numbering), establishing their cis diaxial relationship. In turn, in the spectrum of **8r**, but not in that of **8s**, a nuclear Overhauser effect is observed between the side chain methyl and the hydrogen at C-4, establishing their cis relationship and indicating that the lactone ring may adopt a boat-like conformation in this isomer.
(14) Rance, M. *J. Magn. Reson.* **1987**, *74*, 557–564. Marion, D.; Wüthrich, K. *Biochem. Biophys. Res. Commun.* **1983**, *113*, 967–974.
(15) McKenna, C. E.; Higa, M. T.; Cheung, N. H.; McKenna, M. *Tetrahedron Lett.* **1977**, *2*, 155–158. McKenna, C. E.; Schmidhauser, J. *J. Chem. Soc., Chem. Commun.* **1979**, 739.
(16) Padgette, S. R.; Huynh, Q. K.; Borgmeyer, J.; Shah, D. M.; Brand, L. A.; Re, D. B.; Bishop, B. F.; Rogers, S. G.; Fraley, R. T.; Kishore, G. M. *Arch. Biochem. Biophys.* **1987**, *258*, 564–573.
(17) Mousdale, D. M.; Coggins, J. R. *Planta* **1984**, *160*, 78–83.
(18) Segel, I. H. *Enzyme Kinetics*; John Wiley and Sons: New York, 1975; pp 320–329.
(19) Cleland, W. W. *Methods Enzymol.* **1979**, *63*, 103–138.