Chemical Inhibition of Dehydroquinate Synthase

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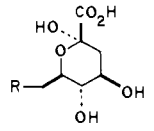
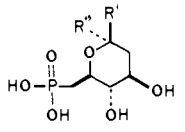
Abstract: Organophosphonate inhibition of substrate 3-deoxy-D-*arabino*-heptulosonic acid 7-phosphate binding to the enzyme dehydroquinate synthase is examined. Enzyme is isolated from *Escherichia coli*. 3-Deoxy-D-*arabino*-heptulosonic acid 7-phosphonate (**1a**) and 3-deoxy-D-*arabino*-heptulosonic acid 7-homophosphonate (**1b**) are used to determine whether isosteric (homophosphonate **1b**) or nonisosteric (phosphonate **1a**) analogues are the best inhibitors. Phosphonate **1a** is a potent competitive inhibitor of dehydroquinate synthase (K_i = 1.1 μM), while no inhibition could be detected with homophosphonate **1b**. The importance of the stereochemical configuration of the carboxylate and the contribution to enzyme inhibition by the hydroxyl group attached to the anomeric carbon are then examined with β-(2,6-anhydro-3-deoxy-D-*arabino*-heptulopyranosid)onate 7-phosphonate (**2a**) and α-(2,6-anhydro-3-deoxy-D-*arabino*-heptulopyranosid)onate 7-phosphonate (**2b**). Anhydro phosphonate **2a** does not inhibit substrate binding to dehydroquinate synthase, while anhydro phosphonate **2b** is a competitive inhibitor (K_i = 129 μM). This inhibition pattern is compared with inhibition of dehydroquinate synthase by β-(2,6-anhydro-3-deoxy-D-*arabino*-heptulopyranosid)onate 7-phosphate (**20a**; K_i = 193 μM) and α-(2,6-anhydro-3-deoxy-D-*arabino*-heptulopyranosid)onate 7-phosphate (**20b**; K_i = 33 μM).

Recognition that chemical disruption of amino acid biosynthesis can lead to plant death has had a profound effect on the design of herbicidal chemicals.[1] For years, discovery of herbicides was predominantly dependent on synthesis of vast numbers of chemical agents followed by screening for plant-killing activity. The decision as to which organic to synthesize has often been based on structural similarities to known herbicides or the novelty of the organic molecule and the chemistry associated with its synthesis. Increasingly, synthetic programs are being directed toward construction of molecules that inhibit specific plant enzymes. Such efforts can take advantage of the large and continually expanding body of chemical synthesis and enzymology associated with amino acid biosynthesis in nonplant systems.

The goal of this account is to synthesize and evaluate the inhibitory characteristics of substrate analogues of dehydroquinate synthase.[2] Dehydroquinate synthase is part of the common pathway[3] of aromatic amino acid biosynthesis in both plants and microbes and catalyzes (Scheme I) the conversion of 3-deoxy-D-*arabino*-heptulosonic acid 7-phosphate (DAHP) to dehydroquinate (DHQ) and inorganic phosphate. All aromatic amino acids and related secondary metabolites must during their biosynthesis pass through dehydroquinate synthase.[4] Putative enzyme inhibitors include 3-deoxy-D-*arabino*-heptulosonic acid 7-phosphonate (**1a**),





1a R = PO_3H_2 **2a** R' = CO_2H, R" = H

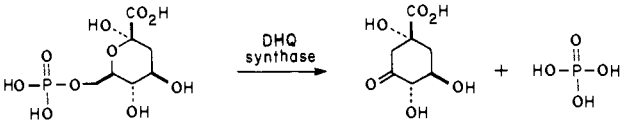
1b R = $CH_2PO_3H_2$ **2b** R' = H, R" = CO_2H

3-deoxy-D-*arabino*-heptulosonic acid 7-homophosphonate (**1b**), β-(2,6-anhydro-3-deoxy-D-*arabino*-heptulopyranosid)onate 7-phosphonate (**2a**), and α-(2,6-anhydro-3-deoxy-D-*arabino*-heptulopyranosid)onate 7-phosphonate (**2b**). Enzyme used for this phase of inhibition studies is purified from *Escherichia coli*. The unique inhibitory pattern that emerges for phosphonate **1a**, homophosphonate **1b**, anhydro phosphonate **2a**, and anhydro phosphonate **2b** is exploited in the accompanying study to determine the impact of dehydroquinate synthase inhibition on plant survival.

Results and Discussion

The inability of 3-deoxy-D-*arabino*-heptulosonic acid (DAH) to serve as a substrate of DHQ synthase indicates the importance

Scheme I



DAHP DHQ

of the phosphate monoester in substrate binding to DHQ synthase. However, an inhibitor of DHQ synthase that incorporates this structural feature would be of scant utility in an intact plant system due to the levels of hydrolyzing phosphatases often encountered in plant tissue.[5] By incorporating a phosphonic acid into a putative inhibitor, the phosphonate moiety would facilitate binding to the enzyme while being inert to the action of phosphatases of plant origin. Two questions then arise: Should the phosphonic acid be isosteric with a methylene group substituted for the oxygen of the DAHP phosphate monoester (homophosphonate **1b**)? Alternatively, should the phosphonic acid be nonisosteric (phosphonate **1a**) with the oxygen of the DAHP phosphate monoester removed?

Isosteric vs Nonisosteric Phosphate Monoester Analogues. As literature molecules first synthesized by Le Marêchal et al.,[2g,6] both **1a** and **1b** provided an ideal test for deriving inhibitors of DHQ synthase from feedstocks produced from engineered auxotrophs of *E. coli*.[7] Successful completion of both syntheses also

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(1) (a) LaRossa, R. A.; Falco, S. C. *Trends Biotechnol.* **1984**, *2*, 158. (b) Hardy, R. W. F.; Giaquinta, R. T. *BioEssays* **1984**, *1*, 152.

(2) (a) Srinivasan, P. R.; Rothschild, J.; Sprinson, D. B. *J. Biol. Chem.* **1963**, *238*, 3176. (b) Rotenberg, S. L.; Sprinson, D. B. *Proc. Natl. Acad. Sci. U.S.A.* **1970**, *67*, 1669. (c) Rotenberg, S. L.; Sprinson, D. B. *J. Biol. Chem.* **1978**, *253*, 2210. (d) Maitra, U. S.; Sprinson, D. B. *J. Biol. Chem.* **1978**, *253*, 5426. (e) Le Marêchal, P.; Azerad, R. *Biochimie* **1976**, *58*, 1123. (f) Le Marêchal, P.; Azerad, R. *Biochimie* **1976**, *58*, 1145. (g) Le Marêchal, P.; Froussios, C.; Level, M.; Azerad, R. *Biochem. Biophys. Res. Commun.* **1980**, *92*, 1104. (h) Frost, J. W.; Bender, J. L.; Kadonaga, J. T.; Knowles, J. R. *Biochemistry* **1984**, *23*, 4470. (i) Widlanski, T. S.; Bender, S. L.; Knowles, J. R. *J. Am. Chem. Soc.* **1987**, *109*, 1873. (j) Bartlett, P. A.; Satake, K. *J. Am. Chem. Soc.* **1988**, *110*, 1628.

(3) (a) Haslam, E. *The Shikimate Pathway*; Wiley: New York, 1974. (b) Weiss, U.; Edwards, J. M. *The Biosynthesis of Aromatic Compounds*; Wiley: New York, 1980. (c) Ganem, B. *Tetrahedron* **1978**, *34*, 3353.

(4) A commercially used, broad-spectrum herbicide (glyphosate) is known to function via inhibition of another enzyme in the common pathway of aromatic amino acid biosynthesis.

(5) See Table I and comments regarding purification of DHQ synthase from *Pisum sativum* in the following paper in this issue.

(6) Le Marêchal, P.; Froussios, C.; Level, M.; Azerad, R. *Carbohydr. Res.* **1981**, *94*, 1.

(7) Reimer, L. M.; Conley, D. L.; Pompliano, D. L.; Frost, J. W. *J. Am. Chem. Soc.* **1986**, *108*, 8010.

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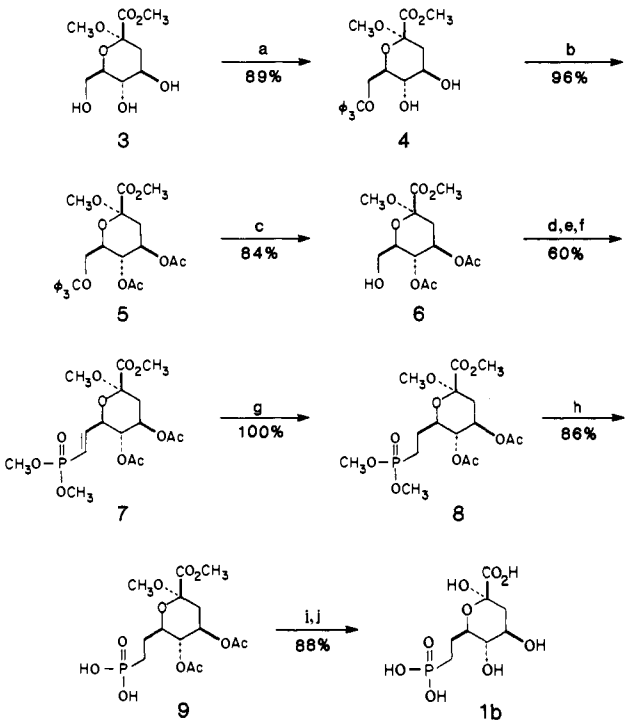
Scheme II[a]



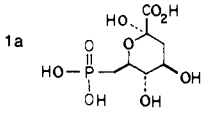
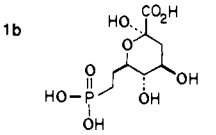
[a] (a) Triphenylmethyl chloride, DMAP, pyridine, DMF, 25 °C; (b) acetic anhydride, DMAP, pyridine, 25 °C; (c) aqueous acetic acid, reflux; (d) pyruvoyl chloride, pyridine, benzene, 25 °C; (e) $h\nu$, benzene, 25 °C; (f) lithium tetramethyl methylenediphosphonate, THF, −20 °C; (g) palladium on carbon, ethanol, TFA, 25 °C; (h) bromotrimethylsilane, 0 °C; (i) 0.10 M NaOH in water/acetonitrile, −5 °C; (j) 0.05 M HCl in water, 50–55 °C.

allowed us to reappraise phosphonates **1a** and **1b** inhibition of *E. coli* DHQ synthase, the first step for ascertaining similarities and differences between inhibition of DHQ synthase isolated from a microbe versus a plant.

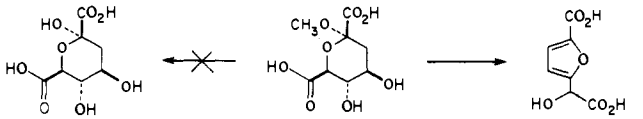
Synthesis of homophosphonate **1b** (Scheme II) began with crystalline methyl (methyl 3-deoxy-D-*arabino*-heptulopyranosid)onate (**3**) derived from DAH. Triphenylmethyl chloride selectively reacted with the C-7 hydroxyl group of **3**. Acetylation of the remaining hydroxyl groups of **4** with acetic anhydride to obtain **5**, was followed by aqueous acid removal of the triphenylmethyl ether. Attempts to oxidize the C-7 alcohol of **6** with Swern conditions[8] led to only small quantities of the desired aldehyde. The majority of product was an α,β-unsaturated aldehyde resulting from initial oxidation of the C-7 alcohol followed by elimination of the acetate attached to C-5. Formation of the elimination product was avoided by exploiting photochemical oxidation.[9] Reaction of **6** with pyruvoyl chloride provided a quantitative yield (by ^{1}H NMR) of the pyruvoyl ester which was photochemically converted to the C-7 aldehyde. The photolysis product was immediately condensed with the lithium salt of tetramethyl methylenediphosphonate[10] yielding **7**. Hydrogenolysis of **7** over palladium on carbon provided a quantitative yield of the fully protected homophosphonate **8**. Stepwise deprotection afforded homophosphonate **1b**.

The determination of the Michaelis constant (K_m = 18 μM) for DAHP binding and the inhibition constant for phosphonate **1a** inhibition (Table I) differed from the values previously reported[2g] by Le Marechal et al. for the *E. coli* enzyme (K_m = 50 μM, K_i = 2.5 μM). These differences could be attributed to the purity of enzyme and assay method used for kinetic measurements. The earlier work used relatively crude, partially purified *E. coli* extract and a coupled enzyme assay. Here, *E. coli* enzyme was homogeneous and enzyme activity measured by direct quantitation of the rate of product inorganic phosphate formation. The largest discrepancy between the two studies is the measured inhibition of DAHP binding by homophosphonate **1b**. Earlier work[2g] indicated a K_i = 260 μM, whereas we observed no inhibition even at 500 μM concentrations of homophosphonate **1b**.

Table I. Inhibition of *Escherichia coli* Dehydroquinate Synthase with Isosteric and Nonisosteric Organophosphonate Analogues of Substrate DAHP

organophosphonate	inhibition	K_i, μM	K_i/K_m
1a 	competitive	1.1	0.061
1b 	no inhibition		

Scheme III



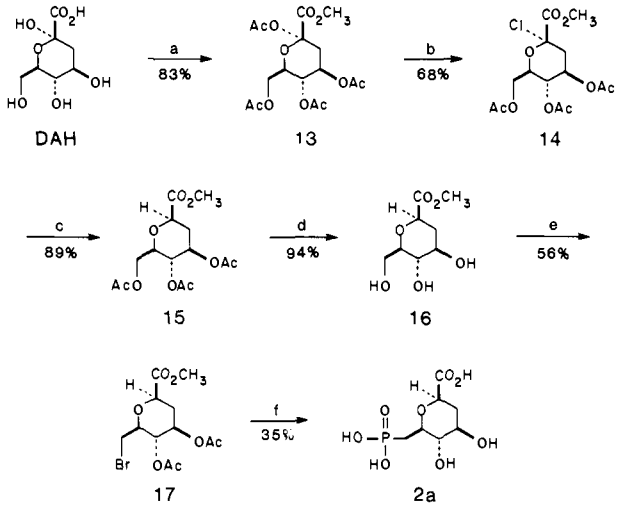
The absence of inhibition of substrate binding by an isosteric homophosphonate analogue is precedented.[11] One explanation may be incomplete dissociation of the phosphonic acid. The dissociation constant of the second proton of a phosphonate moiety is influenced by the proximity of electron-withdrawing groups to the carbon immediately adjacent to the phosphorus.[12] Phosphonate **1a** ($10^{-7.69}$) and homophosphonate **1b** ($10^{-8.18}$) reflect this factor. Nonetheless, the difference in dissociation constants is an inadequate explanation for the potent inhibition observed for phosphonate **1a** relative to homophosphonate **1b** in view of the solution pH and elevated concentrations of homophosphonate employed in the inhibition studies.

Other explanations center on the C–O–P oxygen of the phosphate monoester DAHP. Substitution of a methylene for this oxygen may lead to adverse steric interactions with the enzyme active site, although the position of the methylene group of the homophosphonate **1b** is occupied by the entire phosphonate moiety of phosphonate **1a**. Alternatively, the C–O–P oxygen of DAHP may play an important role as a Lewis base in binding to the active site of DHQ synthase. Such interaction would not be possible with the methylene group of isosteric **1b** but might be possible with the phosphonate moiety of nonisosteric **1a**. At the same time, the phosphonate of nonisosteric **1a** could provide the charge–charge interactions likely essential for binding to the active site.

Circumvention of Furan Formation with Anhydropyranose Analogues. Removal of the methyl ether attached to the anomeric carbon of **1a** and **1b** required similar conditions. Below 50 °C in aqueous solution containing mineral acid, no hydrolysis of the methyl ether is observed. Above 65 °C, deprotection is achieved although a family of resonances in the olefinic region of the ^{1}H NMR arise that cannot be removed by anion-exchange chromatography. These olefinic resonances are likely due to the presence of 2,5-disubstituted furans (similar to furan **12** of Scheme III), whose formation is well documented when 3-deoxy-D-*arabino*-heptulosonic acid (DAH) and the structurally similar

(8) Mancuso, A. J.; Swern, D. *Synthesis* **1981**, 165.
(9) Binkly, R. W. *J. Org. Chem.* **1977**, *42*, 1216.
(10) Roach, D. J. W.; Harrison, R.; Hall, C. R.; Inch, T. D. *Biochem. J.* **1981**, *197*, 735.
(11) Meyer, R. B.; Stone, T. E.; Jesthi, P. K. *J. Med. Chem.* **1984**, *27*, 1095.
(12) (a) Engel, R. *Chem. Rev.* **1977**, *77*, 349. (b) Hori, T.; Horiguchi, M.; Hayashi, A. *Biochemistry of Natural C–P Compounds*; Japanese Association for Research on the Biochemistry of C–P Compounds: Otsu, Shiga, Japan, 1984.

Scheme IV[a]



[a] (a) (i) Acetic anhydride, pyridine, DMAP, 25 °C; (ii) diazomethane, methanol, 25 °C; (b) acetyl chloride, dry HCl, diethyl ether, 0 °C; (c) AIBN, tributyltin hydride, benzene, 75 °C; (d) sodium methoxide, methanol, 25 °C; (e) (i) *N*-bromosuccinimide, triphenylphosphine, DMF, 50 °C; (ii) acetic anhydride, DMAP, pyridine, 25 °C; (f) (i) triethyl phosphite, reflux; (ii) bromotrimethylsilane, 0 °C; (iii) 0.5 N NaOH in water, 0 °C.

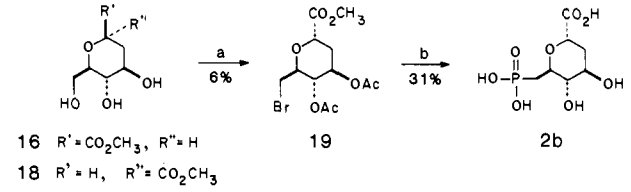
3-deoxy-D-*manno*-octulosonic acid (KDO) are exposed to forcing acidic conditions.[13] With optimization of acid concentration, temperature, and reaction time, the methyl ethers of phosphonate **1a** and homophosphonate **1b** are cleanly removed.

Chemical stability is a key consideration for use of inhibitors of DHQ synthase as in vivo probes of plant metabolism and can be a serious problem for putative inhibitors of DHQ synthase such as 3-deoxy-D-*arabino*-heptulo-1,7-dicarboxylate (**10**; Scheme III). Treatment of precursor methyl pyranoside **11** with acidic conditions optimized for the deprotection of phosphonate **1a** methyl pyranoside afforded none of the desired dicarboxylate **10**. Instead, a quantitative conversion to 2,5-disubstituted furan **12** was observed. Given that furan formation can be quite facile and even dominate the chemistry of DAHP analogues, methods by which furan formation can be circumvented must be evaluated.

Pyranoside ring opening required for furan formation[13a] suggests utilization of a (2,6-anhydro-3-deoxy-D-*arabino*-heptulopyranosid)onate. Locked permanently in the closed ring form, chemical stability should no longer be a problem. On the other hand, enzymological effects attendant with removal of the hydroxyl group attached to the anomeric carbon must be considered. Which anomer, the α- or β-carboxylate, will bind to the active site? What does the anomeric hydroxyl group contribute to inhibitor binding? To resolve these questions, β-(2,6-anhydro-3-deoxy-D-*arabino*-heptulopyranosid)onate 7-phosphonate (**2**) and α-(2,6-anhydro-3-deoxy-D-*arabino*-heptulopyranosid)onate 7-phosphonate (**2b**) were synthesized, and their inhibition of DHQ synthase was determined.

Construction (Scheme IV) of anhydro phosphonate **2a** began with peracetylation of DAH followed by treatment with diazomethane to yield **13**. Treatment of this intermediate with acetyl chloride[14] gave the anomeric chloride **14**. Although the configuration at the anomeric carbon of **14** was not determined, ^{1}H NMR analysis of the product indicated the presence of one diastereomer. Dehalogenation of the C-2 chloride with tributyltin hydride afforded the β-carboxylate **15** as the only product. Removal of the acetates with sodium methoxide in methanol yielded **16**. Selective bromination at C-7, acetylation of the remaining alcohols, and Arbusov condensation with triethyl phosphite followed by deprotection gave anhydro phosphonate **2a**.

Scheme V[a]



[a] (a) (i) *N*-Bromosuccinimide, triphenylphosphine, DMF, 50 °C; (ii) acetic anhydride, DMF, DMAP; (b) (i) triethyl phosphite, reflux; (ii) bromotrimethylsilane, 0 °C; (iii) 0.5 N NaOH in water, 0 °C.

Table II. Inhibition of *Escherichia coli* Dehydroquinate Synthase with Anhydro Organophosphonate Analogues of Substrate DAHP

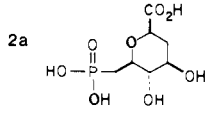
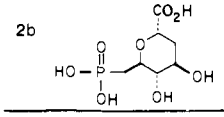
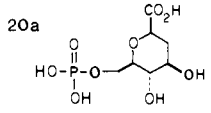
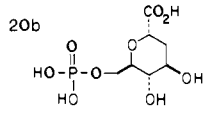
organophosphonate	inhibition	K_i, μM	K_i/K_m
2a 	no inhibition		
2b 	competitive	129	7.2

Table III. Inhibition of *Escherichia coli* Dehydroquinate Synthase with Anhydro Organophosphate Analogues of Substrate DAHP

organophosphate	velocity[a] %	inhibition	K_i, μM
20a 	2	competitive	193
20b 	0	competitive	33

[a] Rate of inorganic phosphate release from anhydropyranose analogue/rate of inorganic phosphate release from substrate DAHP. Conditions and percent velocity value for anhydro-DAHP **20a** taken from ref 2i.

Preparation (Scheme V) of anhydro phosphonate **2b** began with the anomeric mixture (3:1, **16**:**18**) obtained from the precedented hydrolysis of a 1:1 anomeric mixture of α- and β-(2,6-anhydro-3-deoxy-D-*arabino*-hexopyranosid)nitrile followed by treatment with diazomethane.[2i] After bromination and acetylation, the mixture of anomers could be separated to provide pure **19**. Acetylation of the hydroxyl groups using an acetic anhydride/pyridine reaction solution (as in the synthesis of **17**, Scheme IV) resulted in epimerization and a drastic reduction in the yield of the desired α-carboxylate **19**. This apparent preference for the β-carboxylate configuration at C-2 was also reflected in the inability to derive starting material **18** by epimerization under various basic conditions of the β-carboxylate **16**.

Of the anhydro phosphonates, β-(2,6-anhydro-3-deoxy-D-*arabino*-heptulopyranosid)onate 7-phosphonate (**2a**) seemed the most promising as a potential inhibitor of DHQ synthase given the use of β-(2,6-anhydro-3-deoxy-D-*arabino*-heptulopyranosid)onate 7-phosphate (**20a**) as a DHQ synthase substrate.[2i] Obtaining β-(2,6-anhydro-3-deoxy-D-*arabino*-heptulopyranosid)onate **15** (Scheme IV) as the exclusive product during dehalogenation of the anomeric chloride **14** (Scheme IV) meant that substantial quantities of anhydro phosphonate **2a** could be derived from DAH. However, no inhibition of DAHP binding to DHQ synthase could be discerned for anhydro phosphonate **2a** while anhydro phosphonate **2b** was observed to be a competitive inhibitor (Table II).

To obtain additional information as to the anomeric carbon configuration recognized by DHQ synthase, α-(2,6-anhydro-3-

(13) (a) Charon, D.; Szabó, L. *J. Chem. Soc., Perkin Trans. 1* **1973**, 1175. (b) McNicholas, P. A.; Batley, M.; Redmond, J. W. *Carbohydr. Res.* **1987**, *165*, 17.

(14) Bhattacharjee, A. K.; Jennings, H. J.; Kenny, C. P. *Biochemistry* **1978**, *17*, 645.

deoxy-D-*arabino*-heptulopyranosid)onate 7-phosphate (**20b**) was synthesized from **18** (Scheme V). No inorganic phosphate generation indicative of substrate turnover was detected when anhydro-DAHP **20b** was incubated with DHQ synthase for a prolonged (3-h) period of time. Nonetheless, anhydro DAHP **20b** was discovered to be a competitive inhibitor (Table III) of substrate DAHP binding to DHQ synthase. Anhydro-DAHP **20a**, by comparison, is a substantially weaker competitive inhibitor (Table III). Thus the inhibitory pattern observed for anhydro phosphonates **2a** and **2b** is not unique to the substitution of the nonisosteric phosphonate moiety for the phosphate monoester of DAHP.

Inhibition by anhydro phosphonate **2b** and anhydro DAHP **20b** may be due to nonproductive competitive binding to the enzyme active site.[15] Alternatively, DHQ synthase may derive a catalytic advantage by selectively binding the thermodynamically less stable α-carboxylate anomer of DAHP. Binding of the α-carboxylate anomer of DAHP would likely require ring opening and rotation along the C-2, C-3 bond axis to afford the correct stereochemistry at C-1 of product dehydroquinate. α-(2,6-Anhydro-3-deoxy-D-*arabino*-heptulopyranosid)onate 7-phosphonate (**2b**) and DAHP are structurally similar to α-(2,6-anhydro-3-deoxy-D-*manno*-octulopyranosid)onate and KDO, respectively. The α-carboxylate is the anomeric form of KDO that is the substrate for the enzyme CMP-KDO synthase even though the α-carboxylate anomer constitutes only a minor percentage of the pyranose and furanose forms of KDO that exist in aqueous solution.[16a] As with anhydro phosphonates **2a** and **2b**, competitive inhibition of CMP-KDO synthetase is observed with the α-carboxylate but not the β-carboxylate form of anhydro KDO.[16b]

While the stereochemical orientation of the carboxylate attachment to the anomeric carbon of anhydro phosphonates **2a** and **2b** dictates the presence or complete absence of competitive inhibition, removal of the hydroxyl group attached to the anomeric carbon has resulted in a precipitous drop in inhibitory capacity. Other ways of ensuring resistance to furan formation other than removal of the anomeric hydroxyl group will need to be elaborated. Fortunately, the stability of phosphonate **1a** and its potent inhibition of *E. coli* DHQ synthase allows the evaluation of in vitro and in vivo inhibition of plant DHQ synthase to proceed.

Experimental Section

General Chemistry. ^{1}H NMR were recorded on a Varian XL-400 spectrometer, and chemical shifts reported (in parts per million) relative to internal tetramethylsilane (CH_4Si, δ = 0.0) with $CDCl_3$ as solvent and to sodium 3-(trimethylsilyl)propionate-*2,2,3,3-d*$_4$ (TSP, δ = 0.0) when D_2O was the solvent. ^{13}C NMR were recorded on a Varian XL-400 spectrometer and chemical shifts reported (in parts per million) relative to $CDCl_3$ (δ = 77.0) in $CDCl_3$ or internal acetonitrile (CH_3CN, δ = 3.69) in D_2O. ^{31}P NMR were recorded on a Nicolet 300 spectrometer at 121.47 MHz and chemical shifts reported (in parts per million) relative to external orthophosphoric acid (δ = 0.00) in $CDCl_3$ or in D_2O. An IBM IR/98 spectrometer was used for infrared spectra which were recorded in wavenumbers (cm^{-1}). Mass spectra were taken on a Hewlett-Packard 5970 or 5995 spectrometer. Elemental analyses were performed by Microanalytical Laboratories (Stanford, CA) and Desert Analytics (Tucson, AZ). Dissociation constant determinations utilized an Altex Model 3500 digital pH meter fitted with an Orion Research Model 810200 glass electrode. Flash chromatography was carried out on silica gel 60 (40–63 μm, E. Merck) and analytical thin-layer chromatography (TLC) on precoated plates of silica gel 60 F-254 (0.25 mm, E. Merck). TLC plates were visualized by immersion in anisaldehyde stain (by volume: 93% ethanol, 3.5% sulfuric acid, 1% glacial acetic acid, and 2.5% anisaldehyde) followed by heating. Tetramethyl methylenediphosphonate was purchased from Alfa and distilled under reduced pressure. *N*-Bromosuccinimide was recrystallized from water. Dimethylformamide was distilled from magnesium sulfate onto Linde 4A molecular sieves under reduced pressure and stored under nitrogen. Pyridine, dichloromethane, and benzene were distilled from calcium hydride under nitrogen. Tetrahydrofuran was distilled under nitrogen from sodium and benzophenone. All other chemicals were obtained from Aldrich or from Sigma and used without purification unless noted. 3-Deoxy-D-*arabino*-heptulosonic acid 7-phosphate (DAHP) and 3-deoxy-D-*arabino*-heptulosonate 7-phosphonate **1a** were prepared according to literature procedure.[7] DHQ synthase was purified from cell lysate of an overproducing strain of *E. coli*.[2h] Inhibition kinetics followed the conditions and procedures employed with *Pisum* DHQ synthase detailed in the accompanying study.

Methyl (Methyl 7-*O*-trityl-3-deoxy-D-*arabino*-heptulopyranosid)onate (4). Methyl (methyl 3-deoxy-D-*arabino*-heptulopyranosid)onate[7] (**3**; 1.90 g, 8.05 mmol), triphenylmethyl chloride (2.47 g, 8.85 mmol), and *N*,*N*-(dimethylamino)pyridine (0.05 g) were dissolved in 15 mL of dimethylformamide and 5 mL of pyridine under nitrogen. After the resultant mixture was stirred at 25 °C for 18 h, the solvents were removed under reduced pressure and the resulting dark brown syrup was subjected to flash chromatography (hexane/ethyl acetate, 1:1, v/v) to yield 3.43 g (7.16 mmol, 89%) of product **4** as a white solid. Material for analysis was crystallized from hot ethyl acetate as white needles: IR (neat, NaCl) 3617–3124 (br), 3098–3002 (w), 2965–2814 (m), 1750 (s), 1490 (m), 1447 (s), 1267 (m), 1222 (m), 1065 (s), 1047 (s), 987 (m), 912 (m) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.70 (dd, J = 12, 12 Hz, 1 H), 2.36 (dd, J = 5, 13 Hz, 1 H), 3.24 (s, 3 H), 3.42–3.46 (m, 2 H), 3.52–3.61 (m, 2 H), 3.81 (s, 3 H), 3.96–4.00 (m, 1 H), 7.23–7.48 (m, 15 H); ^{13}C NMR ($CDCl_3$) δ 38.8, 50.8, 52.6, 65.2, 69.0, 71.3, 74.4, 87.5, 98.9, 127.2, 128.0, 128.6, 143.5, 168.5; MS, *m/e* (relative intensity) 244 (25), 243 (100), 166 (12), 165 (63), 105 (29). Anal. ($C_{28}H_{30}O_7 \cdot H_2O$) C, H.

Methyl (Methyl 4,5-di-*O*-acetyl-7-*O*-trityl-3-deoxy-D-*arabino*-heptulopyranosid)onate (5). Pyranoside **4** (2.89 g, 6.04 mmol) and *N*,*N*-(dimethylamino)pyridine (0.05 g) were stirred in 20 mL of pyridine and 5 mL of acetic anhydride under a nitrogen atmosphere for 18 h. The solvents were removed under reduced pressure, and the resulting orange oil was subjected to flash chromatography (hexane/ethyl acetate, 4:1, v/v) to yield 3.26 g (5.80 mmol, 96%) of product **5** as a white foam: IR (neat, NaCl) 3111–2811 (m), 1744 (s), 1447 (m), 1363 (m), 1238 (s), 1160 (m), 1060 (s), 902 (m) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.76 (s, 3 H), 1.89 (dd, J = 12, 12 Hz, 1 H), 1.99 (s, 3 H), 2.53 (dd, J = 5, 13 Hz, 1 H), 3.13 (dd, J = 2, 10 Hz, 1 H), 3.23 (dd, J = 6, 10 Hz, 1 H), 3.37 (s, 3 H), 3.84–3.88 (m, 4 H), 5.01 (dd, J = 10, 10 Hz, 1 H), 5.27–5.34 (m, 1 H), 7.21–7.49 (m, 15 H); ^{13}C NMR ($CDCl_3$) δ 20.5, 20.9, 37.1, 50.9, 52.6, 62.9, 69.2, 69.3, 71.2, 86.6, 98.3, 126.9, 127.7, 128.7, 143.7, 167.9, 169.4, 170.0; MS, *m/e* (relative intensity) 303 (6), 259 (7), 258 (13), 244 (25), 243 (100), 165 (60), 127 (21). Anal. ($C_{32}H_{34}O_9$) C, H.

Methyl (Methyl 4,5-di-*O*-acetyl-3-deoxy-D-*arabino*-heptulopyranosid)onate (6). Pyranoside **5** (2.01 g, 3.00 mmol) was dissolved in 40 mL of 80% acetic acid (v/v) and refluxed 10 min. After the mixture was cooled, ethyl acetate was added and the solution made basic with sodium bicarbonate. The organic layer was separated, washed with water followed by saturated sodium chloride, and then dried with magnesium sulfate. The solvent was removed under reduced pressure and the resulting pale yellow oil subjected to flash chromatography (ethyl acetate/hexane, 4:1, v/v) to yield **6** as a white foam (0.96 g, 3.0 mmol, 84%). Material for elemental analysis was recrystallized from ethyl acetate as white needles: IR (neat, NaCl) 3014–2876 (m), 1736 (s), 1717 (s), 1382 (m), 1371 (m), 1270 (m), 1235 (s), 1163 (m), 1109 (m), 1046 (s), 969 (m) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.84 (dd, J = 12, 12 Hz, 1 H), 2.02 (s, 1 H), 2.08 (s, 3 H), 2.53 (dd, J = 5, 13 Hz, 1 H), 3.28 (s, 3 H), 3.65–3.76 (m, 2 H), 3.81 (s, 3 H), 5.01 (dd, J = 10, 10 Hz, 1 H), 5.35–5.42 (m, 1 H); ^{13}C NMR ($CDCl_3$) δ 20.7, 20.9, 37.0, 51.0, 52.8, 61.2, 68.8, 69.0, 72.0, 98.4, 167.8, 170.0, 170.4; MS, *m/e* (relative intensity) 229 (3), 201 (9), 155 (9), 141 (20), 137 (10), 99 (16), 43 (100). Anal. ($C_{13}H_{20}O_9$) C, H.

Methyl (Methyl 4,5-di-*O*-acetyl-3,7,8-trideoxy-7,8(*E*)-didehydro-D-*arabino*-octulopyranosid)onate 8-(Dimethyl phosphonate) (7). Alcohol **6** (0.96 g, 3.0 mmol) was dissolved in 50 mL of benzene with 2.5 mL of pyridine and cooled in an ice bath. Pyruvoyl chloride[17] in benzene was added dropwise until esterification was complete as indicated by TLC. The solution was filtered through a plug of silica gel, and the solvents were removed under reduced pressure. The ester [^{1}H NMR ($CDCl_3$) δ 1.86 (dd, J = 12, 12 Hz, 1 H), 2.02 (s, 3 H), 2.08 (s, 3 H), 2.49 (s, 3 H), 2.53 (dd, J = 5, 13 Hz, 1 H), 3.28 (s, 3 H), 3.81 (s, 3 H), 3.96–4.03 (m, 1 H), 4.31 (dd, J = 2, 10 Hz, 1 H), 4.49 (dd, J = 6, 10 Hz, 1 H), 5.03 (dd, J = 10, 10 Hz, 1 H), 5.32–5.39 (m, 1 H), quantitative yield] was then dissolved in benzene (225 mL) and the flask purged with nitrogen for 1 h. The purge was continued during Pyrex-filtered irradiation with a 450-W medium-pressure Hanovia mercury lamp.[9] Oxidation was monitored by ^{1}H NMR, the reaction being considered complete with the appearance of a doublet at 9.69 ppm and the disappearance of the pyruvoyl methyl at 2.49 ppm. Benzene was removed from the solution

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under reduced pressure, the aldehyde dissolved in 10 mL of THF, and the solution cooled to −20 °C under a nitrogen atmosphere. The anion of tetramethyl methylenediphosphonate (3.05 mmol, generated in THF at −20 °C by the dropwise addition of 3.06 mmol of *n*-butyllithium) was added by cannula.[10] The solution was allowed to warm to room temperature, stirred 12 h, and then extracted from water with three portions of diethyl ether. The resulting brown oil was subjected to flash chromatography (ethyl acetate) to yield 0.76 g (1.8 mmol, 60%) of product **7** as a pale yellow oil: IR (neat, NaCl) 3046–2821 (m), 1747 (s), 1641 (m), 1438 (m), 1373 (m), 1238 (s), 1228 (s), 1170 (m), 1050 (s), 1031 (s), 838 (s) cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.88 (dd, J = 12, 12 Hz, 1 H), 2.01 (s, 3 H), 2.06 (s, 3 H), 2.54 (dd, J = 5, 13 Hz, 1 H), 3.26 (s, 3 H), 3.73 (d, J_{POC} = 11 Hz, 3 H), 3.74 (d, J_{POC} = 11 Hz, 3 H), 3.88 (s, 3 H), 4.25–4.29 (m, 1 H), 4.87 (dd, J = 10, 10 Hz, 1 H), 5.38 (m, 1 H), 6.10 (ddd, J = 2, 17, 18 Hz, 1 H), 6.69 (ddd, J = 5, 17, 22 Hz, 1 H); ^{13}C NMR ($CDCl_3$) δ 20.6, 20.8, 36.9, 52.6 (J_{POC} = 7 Hz, 2 C), 52.7, 68.5, 70.8 (J_{PCCC} = 22 Hz), 71.5, 98.4, 118.6 (J_{PC} = 189 Hz), 145.8 (J_{PCC} = 7 Hz), 167.4, 169.6, 169.9; ^{31}P NMR ($CDCl_3$) δ 20.20; MS, *m/e* (relative intensity) 245 (3), 217 (3), 207 (4), 165 (32), 158 (6), 109 (8), 43 (100). Anal. ($C_{16}H_{25}O_{11}P$) C, H.

Methyl (Methyl 4,5-di-*O*-acetyl-3,7,8-trideoxy-D-*arabino*-octulopyranosid)onate 8-(Dimethyl phosphonate) (8). Phosphonate **7** (0.60 g, 1.4 mmol) was dissolved in 20 mL of ethanol with a drop of added trifluoroacetic acid. After hydrogenation over 10% palladium on carbon in a Parr apparatus for 4 h, the catalyst was removed by filtration through Celite. The solvents were removed under reduced pressure to yield a clear oil, which was subjected to flash chromatography (ethyl acetate), to yield **8** (0.60 g, 1.4 mmol, 100%): IR 3046–2801 (w), 1744 (s), 1644 (m), 1441 (m), 1370 (m), 1241 (s), 1170 (s), 1041 (s) cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.70–1.29 (m, 5 H), 2.01 (s, 3 H), 2.07 (s, 3 H), 2.50 (dd, J = 5, 13 Hz, 1 H), 3.25 (s, 3 H), 3.67–3.71 (m, 1 H), 3.74 (d, J_{POC} = 11 Hz, 6 H), 3.80 (s, 3 H), 4.84 (dd, J = 10, 10 Hz, 1 H), 5.29 (m, 1 H); ^{13}C NMR ($CDCl_3$) δ 19.7 (J_{PC} = 144 Hz), 20.5, 20.6, 24.1, 37.1, 51.0, 52.3 (J_{POC} = 7 Hz, 2 C), 52.6, 68.8, 70.5 (J_{PCCC} = 18 Hz), 71.8, 98.2, 167.7, 169.9; ^{31}P NMR ($CDCl_3$) δ 34.73; MS, *m/e* (relative intensity) 247 (16), 233 (13), 167 (50), 109 (18), 43 (100). Anal. ($C_{16}H_{27}O_{11}P$) C, H.

Methyl (Methyl 4,5-di-*O*-acetyl-3,7,8-trideoxy-D-*arabino*-octulopyranosid)onate 8-Phosphonate (9). Fully protected homophosphonate **8** (0.24 g, 0.56 mmol) was cooled to 0 °C under nitrogen and bromotrimethylsilane (0.34 g, 2.2 mmol) added dropwise.[18] After the mixture stirred for 1 h in an ice batn, excess reagent was removed under reduced pressure and the silyl ester hydrolyzed by stirring with 5 mL of H_2O for 10 min in an ice bath. The solution was extracted once with chloroform and the aqueous portion concentrated under reduced pressure, the temperature being kept below 30 °C, to yield 0.19 g of product **9** (0.48 mmol, 86%) as a white foam: ^1H NMR ($CDCl_3$) δ 1.63–2.05 (m, 5 H), 1.95 (s, 3 H), 2.01 (s, 3 H), 2.45 (dd, J = 5, 13 Hz, 1 H), 3.21 (s, 1 H), 3.65–3.70 (m, 1 H), 3.75 (s, 3 H), 4.80 (dd, J = 10, 10 Hz, 1 H), 5.22–5.28 (m, 1 H); ^{13}C NMR ($CDCl_3$) δ 20.7, 20.9, 21.6 (J_{PC} = 150 Hz), 24.6, 37.2, 51.1, 52.7, 69.0, 71.4 (J_{PCCC} = 17 Hz), 72.4, 98.6, 167.9, 170.0, 170.1.

3,7,8-Trideoxy-D-*arabino*-octulosonic Acid 8-Phosphonate (3-Deoxy-D-*arabino*-heptulosonic Acid 7-Homophosphonate) (1b). Phosphonate **9** (0.22 g, 0.56 mmol) and 50.0 mL of 125 mM aqueous sodium hydroxide with 10.0 mL of acetonitrile were cooled separately in ice baths. The sodium hydroxide solution was added to **9** and stirred at −5 °C for 1 h. Dowex 50 (H^+ form) was then added, making the solution pH <3.0. The entire mixture was loaded onto an additional 10.0 mL of Dowex 50 (H^+ form) and the deprotected phosphonic acid eluted with 50.0 mL of water at 7 °C. The eluant was concentrated under reduced pressure with the temperature kept below 32 °C. The resulting hygroscopic white foam was dissolved in 33.0 mL of 50 mM HCl and stirred at 50–55 °C. After 48 h, the removal of the pyranoside was complete by ^1H NMR, yielding a hygroscopic white foam (0.12 g, 0.42 mmol, 76%): ^1H NMR (D_2O) δ 1.69–2.15 (m, 5 H), 2.70 (dd, J = 5, 13 Hz, 1 H), 3.25 (dd, J = 9, 9 Hz, 1 H), 3.75–3.80 (m, 1 H), 3.88–3.95 (m, 1 H); ^{13}C NMR (D_2O) δ 25.3 (J_{PC} = 137 Hz), 27.0, 41.5, 71.1, 76.0 (J_{PCCC} = 15 Hz), 77.1, 97.7, 175.8; ^{31}P NMR (D_2O) δ 31.90.

Attempted Synthesis of 3-Deoxy-D-*arabino*-heptulo-1,7-dicarboxylate 10. Dicarboxylate **11** (0.48 g, 2.0 mmol) was dissolved in 122 mL of 0.05 N HCl and heated at 60–65 °C. The solution was concentrated under reduced pressure. The resulting clear oil was dissolved in methanol and treated with ethereal diazomethane to yield a white solid that crystallized from hot ethyl acetate, yielding 2,5-disubstituted furan **12**: IR (neat, NaCl) 1777 (s), 1653 (s), 1291 (m), 1033 (m) cm^{-1}; ^1H NMR (D_2O) δ 3.46 (d, J = 6 Hz, 1 H), 3.65 (s, 3 H), 3.73 (s, 3 H), 5.28 (d, J = 6 Hz, 1 H), 6.51 (d, J = 4 Hz, 1 H), 7.17 (d, J = 4 Hz, 1 H); ^{13}C NMR (D_2O) δ 51.8, 53.4, 66.8, 110.1, 118.6, 150.8, 154.3, 158.6, 171.0. Anal. ($C_9H_{10}O_6$) C, H.

Methyl (Acetyl 3,4,5-tri-*O*-acetyl-3-deoxy-D-*arabino*-heptulopyranosid)onate (13). DAH (2.37 g, 11.4 mmol) was dissolved in pyridine (40 mL) and acetic anhydride (30 mL). 4-(Dimethylamino)-pyridine (0.50 g) was added and the mixture stirred for 24 h at room temperature. The solvents were removed under reduced pressure, and the resulting oil was dissolved in chloroform and then extracted twice with cold 5% sulfuric acid. The organic layer was dried (Na_2SO_4) and concentrated to a brown oil. This oil was then dissolved in methanol and treated with ethereal diazomethane. Flash chromatography of the reaction crude (hexane/ethyl acetate, 3:2, v/v) yielded 3.91 g (10.0 mmol, 88%) of product **13** as a crystalline white solid: IR (neat, NaCl) 3020–2890 (w), 1738 (s), 1446 (m), 1365 (m), 1210 (s), 1044 (m) cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.04 (s, 3 H), 2.06 (s, 3 H), 2.09 (s, 3 H), 2.11 (m, 1 H), 2.21 (s, 3 H), 2.70 (dd, J = 5, 13 Hz, 1 H), 3.85 (s, 3 H), 4.06–4.18 (m, 2 H), 4.40 (dd, J = 4, 13 Hz, 1 H), 5.17 (dd, J = 10, 10 Hz, 1 H), 5.35 (m, 1 H); ^{13}C NMR ($CDCl_3$) 20.6, 20.7 (2 C), 20.8, 35.6, 53.3, 61.7, 68.2, 68.4, 71.5, 97.3, 166.4, 168.4, 169.6, 170.1, 170.8. Anal. ($C_{16}H_{22}O_{11}$) C, H.

Methyl β-(2,6-Anhydro-4,5,7-tri-*O*-acetyl-3-deoxy-D-*arabino*-heptulopyranosid)onate (15). Tetra-*O*-acetyl methyl ester **13** (1.47 g, 3.77 mmol) was dissolved in 10 mL of dry ether with 30 mL of freshly distilled acetyl chloride and cooled to 0 °C under nitrogen. Dry HCl gas was bubbled through the reaction solution for 30 min and the reaction stored at 4 °C for 12 h. After nitrogen was bubbled through the solution for 30 min, the reaction mixture was concentrated to an oil. Purification by flash chromatography (hexane/ethyl acetate, 2:1, v/v) provided 0.94 g (2.6 mmol, 68%) of anomeric chloride **14** as a clear oil: IR (neat, NaCl) 3080–2845 (w), 1756 (s), 1436 (m), 1364 (m), 1234 (s), 1174 (s), 1098 (s), 1058 (s), 912 (m) cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.04 (s, 3 H), 2.06 (s, 3 H), 2.09 (s, 3 H), 2.26 (dd, J = 11, 12 Hz, 1 H), 2.91 (dd, J = 5, 13 Hz, 1 H), 3.97 (s, 3 H), 4.15 (dd, J = 2, 12 Hz, 1 H), 4.34 (m, 1 H), 4.44 (dd, J = 4, 13 Hz, 1 H), 5.12 (dd, J = 10, 10 Hz, 1 H), 5.49 (m, 1 H); ^{13}C ($CDCl_3$) δ 20.6 (2 C), 20.7, 40.2, 53.8, 61.1, 67.6, 68.6, 72.7, 95.8, 165.7, 169.6, 169.8, 170.5. Anomeric chloride **14** (0.66 g, 1.8 mmol), tributyltin hydride (0.63 g, 2.2 mmol), and 2,2′-azobis(2-methylpropionitrile) (AIBN; 0.06 g, 0.4 mmol) were dissolved in benzene, and the flask was flushed with nitrogen. The solution was heated at 75 °C for 20 min and then cooled, and the benzene was removed under reduced pressure. Purification by flash chromatography (hexane/ethyl acetate, 2:1, v/v) yielded 0.53 g (1.6 mmol, 89%) of 2,6-anhydro product **15**: IR (neat, NaCl) 3017–2891 (w), 1739 (s), 1447 (m), 1367 (s), 1217 (s), 1045 (m) cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.83 (ddd, J = 12, 12, 12 Hz, 1 H), 2.03 (s, 3 H), 2.04 (s, 3 H), 2.08 (s, 3 H), 2.50 (ddd, J = 2, 5, 6 Hz, 1 H), 3.63–3.67 (m, 1 H), 3.77 (s, 3 H), 4.11–4.17 (m, 2 H), 4.30 (dd, J = 12, 5 Hz, 1 H), 4.98–5.06 (m, 2 H); ^{13}C NMR ($CDCl_3$) δ 20.6, 20.7, 20.8, 33.2, 52.4, 62.4, 68.5, 71.4, 73.0, 76.1, 169.2, 169.6, 170.2, 170.7. Anal. ($C_{14}H_{20}O_9$) C, H.

Methyl β-(2,6-Anhydro-3-deoxy-D-*arabino*-heptulopyranosid)onate (16). Fully protected 2,6-anhydropyranoside **15** (0.23 g, 0.69 mmol) was dissolved in 1 mL of dry methanol, and 1.38 mL of 50 mM sodium methoxide was added dropwise. After being stirred for 15 min, the reaction was neutralized with Dowex 50 (H^+ form) and then loaded onto an additional 2 mL of Dowex 50 (H^+ form). Product was eluted with 6 mL of water. Concentration under reduced pressure yielded 0.13 g (0.65 mmol, 94%) of **16** as a white hygroscopic foam: IR (neat, NaCl) 3650–3000 (br), 1732 (s), 1447 (m) cm^{-1}; ^1H NMR (D_2O) δ 1.62 (ddd, J = 12, 12, 12 Hz, 1 H), 2.40 (ddd, J = 2, 5, 13 Hz, 1 H), 3.31 (dd, J = 9, 9 Hz, 1 H), 3.40–3.44 (m, 1 H), 3.73–3.81 (m, 2 H), 3.92 (dd, J = 2, 13 Hz, 1 H), 3.80 (s, 3 H), 4.33 (dd, J = 2, 12 Hz, 1 H); ^{13}C NMR (D_2O) δ 38.3, 55.7, 63.7, 73.5, 74.1, 76.4, 82.5, 175.7; MS, *m/e* (relative intensity) 207 (1) $M^+ + 1$, 133 (86), 129 (67), 115 (91), 87 (72), 55 (100), 43 (50). Anal. ($C_8H_{14}O_6$) C, H.

Methyl β-(2,6-Anhydro-4,5-di-*O*-acetyl-7-bromo-3-deoxy-D-*arabino*-heptulopyranosid)onate (17). Deprotected 2,6-anhydropyranoside **16** (0.52 g, 2.5 mmol) was dissolved in 20 mL of dry dimethylformamide with *N*-bromosuccinimide (0.899 g, 5.05 mmol) and cooled in an ice bath under a nitrogen stream. Triphenylphosphine (1.32 g, 5.03 mmol) was added over the course of 15 min. The reaction was heated at 50 °C for 2 h and cooled, and 5 mL of methanol was added. After being stirred for 15 min, the solution was concentrated to a syrup and purified by flash chromatography (ethyl acetate) on silica gel to yield a mixture containing succinimide, triphenylphosphine oxide and methyl β-(2,6-anhydro-7-bromo-3-deoxy-D-*arabino*-heptulopyranosid)onate. The above mixture was stirred in 8 mL of pyridine and 2.4 mL of acetic anhydride with catalytic 4-(dimethylamino)pyridine for 18 h. The solvents were removed under reduced pressure, and the resulting orange oil was subjected to flash chromatography (hexane/ethyl acetate, 1:1, v/v) to yield 0.50 g (1.4

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mmol, 56%) of product **17** as a white solid: IR (neat, NaCl) 3638 (w), 3550 (w), 3478 (w), 3100-2870 (br), 1748 (s), 1440 (s), 1372 (s), 1163 (s) cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.85 (ddd, J = 12, 12, 12 Hz, 1 H), 2.04 (s, 3 H), 2.08 (s, 3 H), 2.50 (ddd, J = 2, 5, 13 Hz, 1 H), 3.44 (dd, J = 6, 11 Hz, 1 H), 3.51 (dd, J = 3, 11 Hz, 1 H), 3.79 (s, 3 H), 4.18 (dd, J = 2, 12 Hz, 1 H), 4.95 (dd, J = 9, 9 Hz, 1 H), 5.03-5.09 (m, 1 H); ^{13}C NMR ($CDCl_3$) δ 20.7, 20.8, 30.8, 33.3, 52.5, 71.2, 73.9, 76.7, 77.0, 169.1, 169.6, 170.2; MS, m/e (relative intensity) 355 (9) $M^+ + 2$, 353 (9) M^+, 295 (91), 293 (91), 252 (87), 250 (100), 221 (65), 219 (65), 196 (61), 194 (61). Anal. ($C_{12}H_{17}O_7Br$) C, H.

β-(2,6-Anhydro-3-deoxy-D-*arabino*-heptulopyranosid)onate 7-Phosphonate (2a). Brominated 2,6-anhydropyranoside **17** (0.48 g, 1.4 mmol) was taken up in 5.5 mL of triethyl phosphite and refluxed 24 h under nitrogen. Excess triethyl phosphite was removed under reduced pressure at 50 °C and the resulting dark brown syrup subjected to flash chromatography (hexane/ethyl acetate, 1:2, v/v) to yield a white solid. This solid, dissolved in dichloromethane, was cooled to 0 °C under nitrogen and bromotrimethylsilane (1.21 g, 7.92 mmol) added dropwise. After the resultant mixture was stirred 1 h at 0 °C, excess reagent was removed under reduced pressure and the silyl ester hydrolyzed by vigorous stirring with 10 mL of water for 30 min. The solution was concentrated to an oil and treated with 30.5 mL of 0.5 M aqueous sodium hydroxide for 5 min at 0 °C. The mixture was loaded onto 30 mL of Dowex 50 (H^+ form) and the product eluted with 60 mL of water at 4 °C. The eluant was concentrated under reduced pressure, loaded onto 10 mL of AG 1 × 8 equilibrated with 0.20 M sodium acetate at 4 °C, and washed with 10 mL of 0.20 M sodium acetate, followed by elution of the phosphonic acid with a linear gradient of (100 mL + 100 mL, 0.20-2.0 M) sodium acetate, pH 4.75. The fractions were assayed and those containing phosphonic acid pooled, passed down 200 mL of Dowex 50 (H^+ form), and then eluted with 300 mL of water. The water was removed under reduced pressure, during which the temperature was kept below 30 °C to yield 0.041 g (0.16 mmol, 35%) of **2a** as a hygroscopic foam: ^1H NMR (D_2O) δ 1.64 (ddd, J = 12, 12, 12 Hz, 1 H), 1.94-2.08 (m, 1 H), 2.31-2.41 (m, 2 H), 3.16 (dd, J = 9, 9 Hz, 1 H), 3.60 (ddd, J = 10, 10, 10 Hz, 1 H), 3.73-3.79 (m, 1 H), 4.28 (dd, J = 2, 12 Hz, 1 H); ^{13}C NMR (D_2O) δ 32.3 (J_{PC} = 137 Hz), 38.5, 73.8, 76.5, 77.6 (J_{PCC} = 15 Hz), 77.9, 177.1; ^{31}P NMR (D_2O) δ 27.43. Anal. ($C_7H_{13}O_8P \cdot H_2O$) C, H.

Methyl α-(2,6-Anhydro-7-bromo-3-deoxy-D-*arabino*-heptulopyranosid)onate (19). An anomeric mixture of methyl α- and β-(2,6-anhydro-3-deoxy-D-*arabino*-heptulopyranosid)onates (**16** and **18**; 2.5 g, 12 mmol) was dissolved in 100 mL of dry dimethylformamide with *N*-bromosuccinimide (4.4 g, 25 mmol) and cooled in an ice bath under a nitrogen stream. Triphenylphosphine (6.4 g, 25 mmol) was added over the course of 30 min. The reaction was heated at 50 °C for 2 h and cooled and 25 mL of methanol added. After being stirred for 15 min, the solution was concentrated to a syrup and purified by flash chromatography (ethyl acetate) on silica gel to yield a mixture containing succinimide, triphenylphosphine oxide, and an anomeric mixture of methyl (2,6-anhydro-7-bromo-3-deoxy-D-*arabino*-heptulopyranosid)onate. The above mixture was stirred in 30 mL of dimethylformamide and 12 mL of acetic anhydride with catalytic 4-(dimethylamino)pyridine for 18 h. The solvents were removed under reduced pressure, and the resulting orange oil was subjected to flash chromatography (hexane/ethyl acetate, 3:1, v/v) to yield 0.22 g (0.68 mmol, 6% based on the anomeric mixture) of white, solid **19**: IR (neat, NaCl) 2951 (w), 1745 (s), 1433 (m), 1372 (m), 1235 (s), 1143 (m), 1050 (s) cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.02-2.11 (m, 1 H), 2.04 (s, 3 H), 2.06 (s, 3 H), 2.51 (ddd, J = 3, 5, 13 Hz, 1 H), 3.43 (dd, J = 5, 11 Hz, 1 H), 3.57 (dd, J = 3, 11 Hz, 1 H), 4.15-4.20 (m, 1 H), 4.61 (dd, J = 2, 6 Hz, 1 H), 4.97 (dd, J = 9, 9 Hz, 1 H), 5.00-5.06 (m, 1 H); ^{13}C NMR ($CDCl_3$) δ 20.7, 20.9, 31.1, 31.6, 52.5, 69.0, 70.9, 71.0, 73.1, 169.6, 170.0, 170.7; MS, m/e (relative intensity) 213 (10), 175 (16), 173 (16), 170 (21), 43 (100). Anal. ($C_{12}H_{17}O_7Br$) C, H.

α-(2,6-Anhydro-3-deoxy-D-*arabino*-heptulopyranosid)onate 7-Phosphonate (2b). Intermediate **19** was converted to **2b** by using the conditions and reagents described for anhydro phosphonate **2a** synthesis from **17**. ^1H NMR (D_2O) δ 1.88 (ddd, J = 6, 12, 14 Hz, 1 H), 2.02-2.15 (m, 1 H), 2.32-2.41 (m, 1 H), 2.47 (ddd, J = 2, 5, 14 Hz, 1 H), 3.24 (dd, J = 9, 9 Hz, 1 H), 3.60-3.66 (m, 1 H), 3.80 (ddd, J = 3, 9, 21 Hz, 1 H), 4.64 (dd, J = 2, 6 Hz, 1 H); ^{13}C NMR (D_2O) δ 32.4 (J_{PC} = 138 Hz), 35.7, 71.7, 74.7, 75.7 (J_{PCCC} = 6 Hz), 77.4 (J_{PCC} = 13 Hz), 177.6; ^{31}P NMR (D_2O) δ 27.40. Anal. ($C_7H_{13}O_8P \cdot H_2O$) C, H.

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Probing Lethal Metabolic Perturbations in Plants with Chemical Inhibition of Dehydroquinate Synthase

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Abstract: Plant dehydroquinate synthase is purified from *Pisum sativum*. Inhibition of substrate 3-deoxy-D-*arabino*-heptulosonic acid 7-phosphate binding to enzyme is determined with 3-deoxy-D-*arabino*-heptulosonic acid 7-phosphonate (**1a**), 3-deoxy-D-*arabino*-heptulosonic acid 7-homophosphonate (**1b**), β-(2,6-anhydro-3-deoxy-D-*arabino*-heptulopyranosid)onate 7-phosphonate (**2a**), and α-(2,6-anhydro-3-deoxy-D-*arabino*-heptulopyranosid)onate 7-phosphonate (**2b**). Competitive inhibition of *Pisum* dehydroquinate synthase is observed with phosphonate **1a** (K_i = 0.8 μM) and anhydro phosphonate **2b** (K_i = 296 μM). Phosphonate **1b** and anhydro phosphonate **2a** do not inhibit substrate binding to *Pisum* dehydroquinate synthase. A range of plant species (*P. sativum*, *Echinochloa crusgalli*, *Setaria viridis*, *Sorghum halepense*, and *Avena fatua*) is then treated with the organophosphonate (**1a**) found to be the most potent inhibitor of plant dehydroquinate synthase. An increase in dephosphorylated substrate of dehydroquinate synthase serves as a marker of in vivo enzyme inhibition, while visual and growth indexes are used to gauge herbicidal activity. Both DAH buildup and herbicidal effects are observed for several of the plant species upon postemergent exposure to phosphonate **1a**.

Elaboration of the mechanism of action of the broad-spectrum herbicide *N*-phosphonomethylglycine (glyphosate)[1] has played an important role in the ongoing evolution of strategies employed in herbicide identification. There can be little doubt that EPSP synthase, an enzyme of the common pathway of aromatic amino acid biosynthesis (Scheme I), is the primary target of glyphosate.[2] Yet it is not simply enzyme inhibition but rather the impact of

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(2) Three lines of evidence support this view. These include shikimate buildup,[3] EPSP synthase inhibition,[4] and mutation and overproduction of EPSP synthase which result in resistance to glyphosate.[5]

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