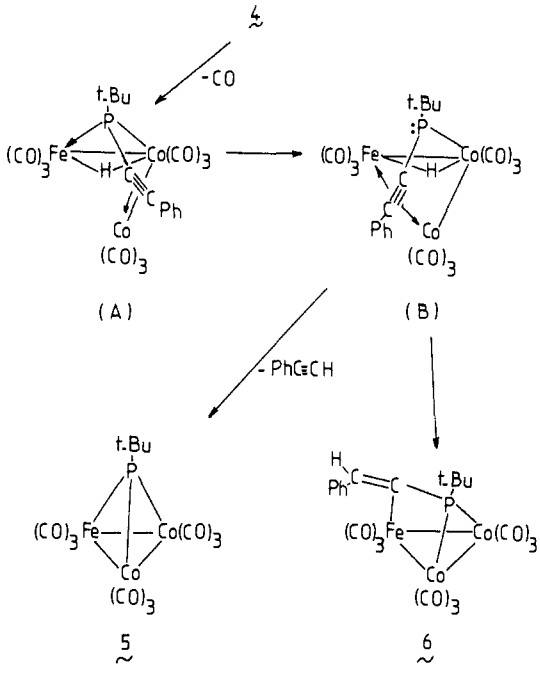
Scheme I



Crystal data and data collection parameters are listed in Table II. Intensities of two standard reflections were monitored every hour. They showed no variation during data collection. Corrections were made for Lorentz and polarization effects. ψ scan curves showed significant intensity variations. Absorption corrections were then applied via an empirical correction technique.[17]

Computations were performed using the CRYSTALS system[18] adapted on a VAX 11/725. Atomic scattering factors for neutral Fe, Co, P, C, O, and H were taken from the International Tables for Crystallography.[19] Anomalous dispersion for Fe and Co atoms were taken into account.

The positions of Fe and Co atoms were determined by Harker vector analysis of three-dimensional Patterson maps. All remaining non-hydrogen atoms were found by successive electron density map calculations. All non-hydrogen atoms were refined anisotropically, except phenyl rings, which were refined as isotropic rigid groups (C-C = 1.39 Å). Hydrogen atoms were located on a difference electron density map. Their atomic coordinates were refined with a fixed overall isotropic thermal parameter for two rounds of least-squares refinement with fixed parameters for heavy atoms. They were not further refined in the final stages of calculations. The criteria for a satisfactory completed analysis were the ratio of parameter shifts to standard deviations (Table II) and no significant features in the final difference map. Atomic coordinates are given in Table III.

Supplementary Material Available: A listing of anisotropic thermal parameters and hydrogen atomic coordinates (3 pages); tables of observed structure amplitudes and structure factors calculated from the final atomic parameters (12 pages). Ordering information is given on any current masthead page.

(17) North, A. C. T.; Phillips, D. C.; Mathews, F. S. *Acta Crystallogr., Sect. A: Cryst. Phys., Diffr., Theor. Gen. Crystallogr.* **1968**, *A24*, 351-359.

(18) Carruthers, B.; Watkin, D. W. J. CRYSTALS, *an Advanced Crystallographic Program System*; Chemical Crystallography Laboratory: University of Oxford, England, 1984.

(19) *International Tables for X-Ray Crystallography*; Kynoch: Birmingham, England, 1974; Vol. IV, pp 99-107.

Construction of an Enzyme-Targeted Organophosphonate Using Immobilized Enzyme and Whole Cell Synthesis

L. M. Reimer, D. L. Conley, D. L. Pompliano, and J. W. Frost*

Contribution from the Department of Chemistry, Stanford University, Stanford, California 94305. *Received November 1, 1985*

Abstract: A potential inhibitor of plant aromatic amino acid biosynthesis, 3-deoxy-D-*arabino*-heptulosonic acid 7-phosphonate, is constructed. The centerpiece of the convergent strategy is methyl (methyl 3-deoxy-D-*arabino*-heptulopyranosid)onate. This intermediate is derived from 3-deoxy-D-*arabino*-heptulosonic acid 7-phosphate produced by immobilized enzyme synthesis or via 3-deoxy-D-*arabino*-heptulosonic acid which results from the whole cell synthesis. By the integration of organic chemical synthesis with immobilized enzyme or whole cell synthesis, the desired product organophosphonate can be conveniently made from D-fructose or D-glucose, respectively.

Disruption of amino acid biosynthesis in plants is purported to be an extremely effective vehicle for herbicide action.[1-3] The historical roots for this notion can be found in herbicidal disruption of plant aromatic amino acid biosynthesis (the shikimate pathway). Inhibition and inactivation of shikimate-pathway enzymes continues to be the focus of widespread interest in the development of specific, enzyme-targeted herbicides. Derivatives of metabolites intermediate in the common pathway offer promise as effective enzyme inhibitors. Like the naturally occurring metabolites, such putative herbicidal agents can be constructed from simple carbohydrates.

The appeal of enzyme-targeted herbicides derivable from carbohydrates must be tempered by the complexity of the chemical syntheses demanded by such an approach. In this report the construction of 3-deoxy-D-*arabino*-heptulosonic acid 7-phosphonate (DAH phosphonate, **1**)[4] is detailed. Synthetic organic, immobilized enzyme, and whole cell syntheses are exploited to achieve

(1) For a useful overview, see: Hardy, R. W. F.; Giaquinta, R. T. *BioEssays* **1984**, *1*, 152.

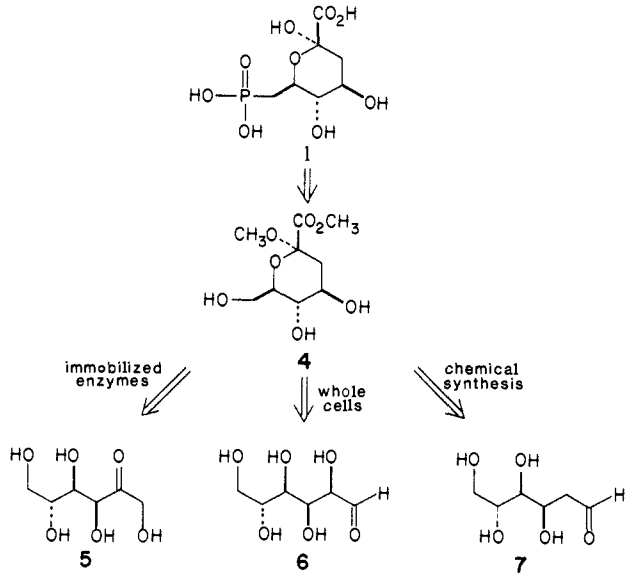
(2) Disruption of aromatic amino acid biosynthesis: (a) Jaworski, E. G. *J. Agric. Food Chem.* **1972**, *20*, 1195. (b) Amrhein, N.; Holländer, H. *Naturwissenschaften* **1981**, *68*, 43. (c) Holländer-Czytko, H.; Amrhein, N. *Plant Sci. Lett.* **1983**, *29*, 89. (d) Amrhein, N.; Schab, J.; Steinrücken, H. C. *Naturwissenschaften* **1980**, *67*, 356. (e) Steinrucken, H. C.; Amrhein, N. *Biochem. Biophys. Res. Commun.* **1980**, *94*, 1207. (f) Amrhein, N.; Johanning, D.; Schab, J.; Schulz, A. *FEBS Lett.* **1983**, *157*, 191. (g) Boocock, M. R.; Coggins, J. R. *FEBS Lett.* **1983**, *154*, 127. (h) Mousedale, D. M.; Coggins, J. R. *Planta* **1984**, *160*, 78.

(3) Disruption of branched-chain amino acid biosynthesis: (a) LaRossa, R. A.; Schloss, J. V. *J. Biol. Chem.* **1984**, *259*, 8753. (b) Chaleff, R. S.; Mauvais, C. J. *Science (Washington, D. C.)* **1984**, *224*, 1443. (c) Ray, T. B. *Plant Physiol.* **1984**, *75*, 827. (d) Rost, T. L.; Reynolds, T. *Plant Physiol.* **1985**, *77*, 481. (e) LaRossa, R. A.; Smulski, D. R. *J. Bacteriol.* **1984**, *160*, 391. (f) Schloss, J. V.; Van Dyk, D. E.; Vasta, J. F.; Kutny, R. M. *Biochemistry* **1985**, *24*, 4952. (g) Shaner, D. L.; Anderson, P. C.; Stidham, M. A. *Plant Physiol.* **1984**, *76*, 545.

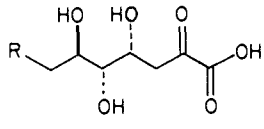
(4) DAH phosphonate is a literature molecule although no spectroscopic information is available: Le Marechal, P.; Froussios, C.; Level, M.; Azerad, R. *Carbohydr. Res.* **1981**, *94*, 1.

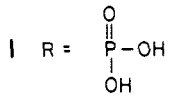
0002-7863/86/1508-8010$01.50/0

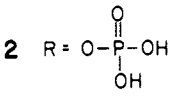
Scheme I



a practical means for obtaining DAH phosphonate from simple carbohydrates.







3 R = OH

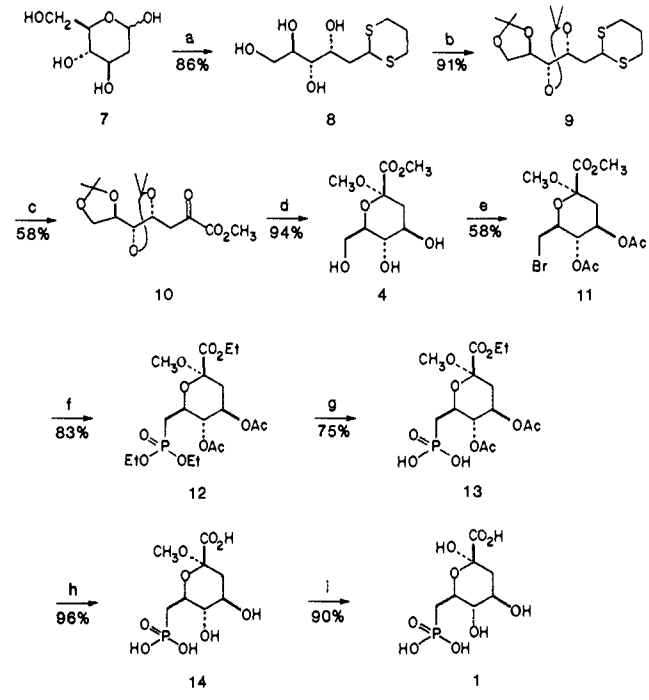
Immobilized enzyme synthesis is now a common feature of the chemical literature while whole cell synthesis has long been a mainstay of the pharmaceutical industry. Both methodologies are well suited for the preparative-scale production of moles of material. Yet often in the identification of biocidal agents, swift construction of a wide range of organics is important on a laboratory scale of 1–10 mmol of material. How can immobilized enzyme and whole cell syntheses expedite this task? The construction of DAH phosphonate provides an opportunity to examine the merits of these two methodologies when combined with synthetic organic chemistry.

Results and Discussion

DAH phosphonate is intended to be an effective inhibitor of substrate 3-deoxy-D-*arabino*-heptulosonic acid 7-phosphate (DAHP, **2**) binding to the aromatic amino acid biosynthetic enzyme, dehydroquinate (DHQ) synthetase.[5] Selection of DAH phosphonate as a synthetic target followed from ongoing efforts to purify the enzyme DHQ synthetase from pea (*Pisum sativum* cv. progress 9).[6] DAH phosphonate is similar to the native substrate, DAHP, in that it is a heptulosonic acid. The phosphonic acid moiety was expected to be an excellent analogue for the phosphate group of DAHP with the added advantage that DAH phosphonate would be immune to hydrolysis by phosphatases of plant origin. Lastly, the carbon to phosphorus bond of DAH phosphonate is a likely handle for eventual biodegradation by soil microbes.[7]

(5) Le Marechal, P.; Froussios, C.; Level, M.; Azerad, R. *Biochem. Biophys. Res. Commun.* **1980**, *92*, 1104.
(6) Pompliano, D. L.; Reimer, L. M.; Frost, J. W., unpublished results.

Scheme II[a]



[a] (a) 1,3-Propanedithiol, concentrated HCl, EtOH, 25 °C. (b) Acetone, catalytic concentrated sulfuric acid, 25 °C. (c) (i) *n*-Butyllithium, THF, −40 °C; (ii) methyl chloroformate, −78 °C; (iii) *N*-bromosuccinimide, 5% water in acetone, 0 °C. (d) 1 M HCl in methanol, reflux. (e) (i) *N*-Bromosuccinimide, triphenylphosphine, dimethylformamide, 25 °C; (ii) acetic anhydride, pyridine, 25 °C. (f) Triethyl phosphite, reflux. (g) (i) bromotrimethylsilane, 0 °C; (ii) water, 25 °C. (h) 0.5 N NaOH in water, 0 °C. (i) 0.025 N HCl in water, 50 °C.

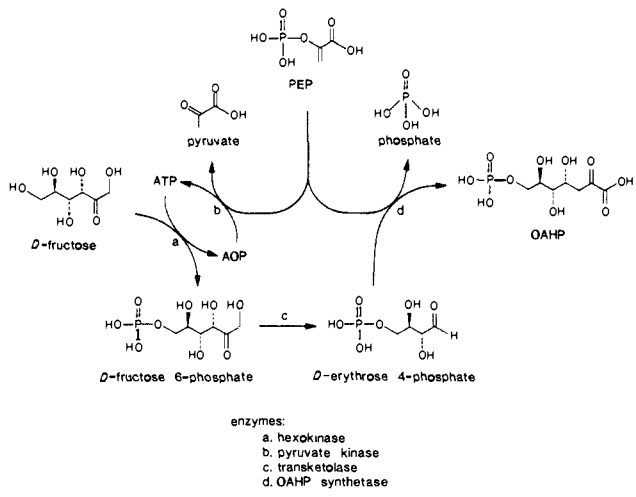
Retrosynthetic analysis of DAH phosphonate (Scheme I) indicates two distinct challenges: construction of the seven-carbon heptulopyranosidonate **4** and elaboration of this carbohydrate into the enzyme-targeted organophosphonate **1**. Methyl (methyl 3-deoxy-D-*arabino*-heptulopyranosid)onate **4** can be obtained from D-fructose **5** with immobilized enzyme synthesis of 3-deoxy-D-*arabino*-heptulosonic acid 7-phosphate (**2**) or from D-glucose **6** by using whole cell synthesis of 3-deoxy-D-*arabino*-heptulosonic acid (**3**). Alternatively, the key intermediate can be chemically synthesized from 2-deoxy-D-glucose (**7**).

Initially, our main interest was obtaining DAH phosphonate in as expeditious a fashion as possible. The precedented chemical synthesis of intermediate **4** from 2-deoxy-D-glucose offered the advantage of speed.[8] Steps a–c of Scheme II summarize the transformations necessary for the synthesis of fully protected 3-deoxy-D-*arabino*-heptulosonate **10**. Reaction of **10** in refluxing acidic methanol followed by crystallization affords **4**. A number of the physical characteristics of methyl (methyl 3-deoxy-D-*arabino*-heptulopyranosid)onate (**4**) make it an ideal intermediate. These include its stability and effective purification by recrystallization.

Introduction of the phosphonic acid moiety begins with selective bromination of the C-7[9] carbon of **4** followed by acylation of the remaining free hydroxyl groups at C-4 and C-5 to yield **11**. Standard Arbusov methodology smoothly converts **11** into the fully protected DAH phosphonate **12**. Triethyl phosphite is used as the solvent for the condensation, during which transesterification leads to the ethyl ester **12**. Reaction with bromotrimethylsilane[10]

(7) See: Cordeiro, M. L.; Pompliano, D. L.; Frost, J. W. *J. Am. Chem. Soc.* **1986**, *108*, 332 and references therein.
(8) Frost, J. W.; Knowles, J. R. *Biochemistry* **1984**, *23*, 4465.
(9) Hanessian, S.; Ponpipom, M. M.; Lavalee, P. *Carbohydr. Res.* **1972**, *24*, 45.
(10) McKenna, C. E.; Higa, M. T.; Cheung, N. H.; McKenna, M. C. *Tetrahedron Lett.* **1977**, 155.

Scheme III



and hydrolysis of the resulting bis(trimethylsilyl) ester produced the free phosphonic acid moiety on **13**. Brief treatment of **13** with 0.5 N sodium hydroxide at 0 °C hydrolyzed the remaining esters. All of the protecting groups which remain on **13** could conceivably be removed by acid hydrolysis. Although appealing, such an approach was avoided so as to focus on the troublesome removal of the methyl ether. Optimized conditions for the conversion of **14** to DAH phosphonate **1** required careful control of the temperature, reaction time, and acid concentration.

Inspection of the total synthesis of Scheme II indicates that a great deal of the manipulations are associated with assembly of the heptulosonate backbone. Much of our time was spent preparing quantities of **4** rather than examining the organic chemistry subsequent to the synthesis of this intermediate. Furthermore, the starting carbohydrate 2-deoxy-D-glucose (**7**) due to its expense[11] is a rather poor choice of a carbohydrate upon which to base an extensive synthetic program. Although **7** can be made from D-glucose,[12] the route is time and labor intensive.

At this juncture we turned to some basic elements of biotechnology to remedy the cumbersome nature of the purely chemical synthesis of DAH phosphonate from carbohydrate precursors. An immobilized enzyme system appeared attractive due to the well-documented ability of such an approach[13] to accomplish in a single reaction vessel what normally would require a multitude of synthetic organic steps. The pronounced stabilization of enzymes when immobilized also offered the promise that once the immobilized enzyme "reactor" was assembled it could be reused multiple times. Construction of such a system (Scheme III) followed a careful examination of how nature biosynthesizes DAHP.

Condensation of D-erythrose 4-phosphate (E-4-P) with phosphoenolpyruvate (PEP) catalyzed by the enzyme DAHP synthetase constitutes the first committed step of aromatic amino acid biosynthesis. Use of this enzyme for convenient access to shikimate-pathway metabolites is limited by the chemical characteristics of E-4-P.[14] E-4-P is notorious for its propensity toward dimerization, isomerization, and elimination, which sets strict limits on the pH, temperature, and dilution at which it can be manipulated. Existing routes for obtaining E-4-P use either a degradation of D-glucose 6-phosphate[15a] or a hydrogenolysis of the cyanohydrin of D-glyceraldehyde 3-phosphate.[15b] The route from D-glucose 6-phosphate yields antipodally clean E-4-P but is a tedious procedure due to the experimental requirements of the lead(IV) tetraacetate oxidation. Use of D-glyceraldehyde 3-phosphate is a better protocol but requires additional chromatography to separate the C-2 epimers formed during the cyanohydrin formation. Neither protocol avoids the necessity of handling the sensitive aldose phosphate.

The reactor design of Scheme III circumvents these difficulties by substituting enzymatic for manual manipulations of E-4-P. In the first step, D-fructose is converted to D-fructose 6-phosphate with phosphate transfer from adenosine triphosphate (ATP) catalyzed by immobilized hexokinase. Immobilized transketolase then removes C-1 and C-2, generating E-4-P[16] which is immediately condensed with PEP in a reaction mediated by immobilized DAHP synthetase. Due to the affinity of DAHP synthetase for E-4-P,[17] the steady-state concentration of the troublesome aldose is kept low. While the reactor is in operation the E-4-P concentration is below the assay detection limit.[18] Thus immobilized enzyme synthesis offers the critical advantage of allowing E-4-P to be conveniently generated and reacted within a time frame short enough to preclude side reactions.

In the initial conversion of D-fructose to D-fructose 6-phosphate, only catalytic levels of ATP are used. As it is formed, the adenosine diphosphate (ADP) is recycled to ATP by phosphate transfer from PEP catalyzed by pyruvate kinase.[19] PEP also provides C-1 through C-3 of DAHP in the last step of the reaction involving DAHP synthetase. Thus PEP essentially controls the enzyme reactor. Starting material is driven into the reactor via the energetically favorable reaction with PEP catalyzed by pyruvate kinase while E-4-P is forced irreversibly forward to product after condensation with PEP catalyzed by DAHP synthetase. With these design elements an 85% conversion of D-fructose to DAHP is achieved in a single reaction vessel.

Cofactors which are needed in immobilized enzyme synthesis of DAHP include thiamine pyrophosphate and ATP for the enzymes transketolase and pyruvate kinase, respectively. Thiamin pyrophosphate is not consumed during the course of the immobilized enzyme synthesis, and only micromolar concentrations of the material are needed. Likewise, only catalytic amounts of ATP are required as it is recycled by pyruvate kinase. Cosubstrates such as D-ribose 5-phosphate[20] and PEP[21] can be easily prepared in large quantities with Whitesides' methodology.

Typically, there is an 80–90% recovery of activity upon immediate reuse of the reactor of Scheme III. The immobilized enzymes can be recycled by centrifugation and resuspension in the substrate, cosubstrate, and cofactor solution. Prolonged storage of the centrifuged mixture of immobilized enzymes leads to substantially lowered DAHP yields upon reuse. The problem can be traced directly to DAHP synthetase, which loses activity when stored over a period of days at 4 °C. The somewhat disappointing catalytic lifetime of immobilized DAHP synthetase is not a critical weakness as the enzyme is available from overproducing strains of *Escherichia coli*,[22] and its purification and immobilization are quite straightforward.[17] Transketolase is not currently available in an overproducing strain of microbe, and its cost from commercial sources is substantial. Fortunately, transketolase possesses noteworthy stability when immobilized. Over a 9-month period of storage in distilled, deionized water at 4 °C, there was no loss of activity of the immobilized transketolase.

Integration of the immobilized enzyme synthesis with the chemical synthesis requires hydrolysis of product DAHP to DAH

(11) 2-Deoxy-D-glucose costs approximately 1000 times the cost of comparably pure D-glucose or D-fructose.
(12) Bolliger, H. R. In *Methods in Carbohydrate Chemistry*; Whistler, R. L., Wolfrom, M. L., Eds.; Academic: New York, 1962; p 186.
(13) Whitesides, G. M.; Wong, C.-H. *Aldrichimica Acta* **1983**, *16*, 27.
(14) (a) Blackmore, P. F.; Williams, J. F.; MacLeod, J. K. *FEBS Lett.* **1976**, *64*, 222. (b) Williams, J. F.; Blackmore, P. F.; Duke, C. C.; MacLeod, J. K. *J. Biochem. (Tokyo)* **1980**, *12*, 339. (c) Duke, C. C.; MacLeod, J. K.; Williams, J. F. *Carbohydr. Res.* **1981**, *95*, 1.
(15) (a) Sieben, A. S.; Perlin, A. S.; Simpson, F. J. *Can. J. Biochem.* **1966**, *44*, 663. (b) Serianni, A. S.; Pierce, J.; Barker, R. *Biochemistry* **1979**, *18*, 1192.
(16) The two-carbon fragment is transferred to D-ribose 5-phosphate.
(17) Schoner, R.; Herrmann, K. M. *J. Biol. Chem.* **1976**, *251*, 5440.
(18) Paoletti, F.; Williams, J. F.; Horecker, B. L. *Anal. Biochem.* **1979**, *95*, 250.
(19) Nageswara Rao, B. D.; Kayne, F. J.; Cohn, M. *J. Biol. Chem.* **1979**, *254*, 2689.
(20) Gross, A.; Abril, O.; Lewis, J. L.; Geresh, S.; Whitesides, G. M. *J. Am. Chem. Soc.* **1983**, *105*, 7428.
(21) Hirschbein, B. L.; Mazenod, F.; Whitesides, G. M. *J. Org. Chem.* **1982**, *47*, 3765.
(22) Ogino, T.; Garner, C.; Markley, J. L.; Herrmann, K. M. *Proc. Natl. Acad. Sci. U.S.A.* **1982**, *79*, 5828.

followed by the conversion of the DAH to intermediate **4**. This is done by adding immobilized alkaline phosphatase to the crude supernatant of the centrifugated enzyme reactor. Subsequent to the complete hydrolysis of DAHP, immobilized phosphatase was recovered by centrifugation and product DAH isolated after chromatography on an anion-exchange column. Intermediate **4** was obtained after refluxing the DAH in acidic methanol and allowing the desired product to crystallize from the neutralized, decolorized reaction solution.

Immobilized enzyme synthesis unquestionably constitutes the best route to DAHP. The percent conversion from simple carbohydrates is in excess of 1 order of magntiude higher than that accessible with any other approach.[8,23] Purification of the DAHP produced by immobilized enzyme synthesis is straightforward and provides product which is pristinely pure. The reactor allows intermediate **4** to be derived from a simple carbohydrate and reduces the number of purely synthetic organic operations necessary to obtain **4**. Unfortunately, many of these synthetic organic steps have been replaced by the various operations needed to assemble the components of the immobilized enzyme system and convert product DAHP to a synthetically useful form. Although this somewhat subtracts from its utility in amassing quantities of intermediate **4**, the immobilized enzyme synthesis is a useful alternative to total chemical synthesis of **4**.

Whole cell synthesis is yet another option. Procaryotes which lack specific enzymes in aromatic amino acid biosynthesis are auxotrophic, requiring in their growth media the presence of the aromatic amino acids which they are unable to synthesize. For many of the mutants which lack enzymes of the common pathway of aromatic amino acid biosynthesis, the substrate of the missing enzyme accumulates in the supernatant of the growth media. This extremely useful characteristic of auxotrophs was instrumental in the chemical identification of the intermediates in the shikimate pathway.[24]

DAH and DAHP accumulate in the growth mixture of *E. coli* JB-5 which lacks dehydroquinate synthetase due to the absence of a functional *aroB* gene. The amount of heptulosonate excreted by *E. coli* JB-5 is less than that produced in the supernatant of the immobilized enzyme system.[25a] However, the vast majority of the heptulosonate produced by the *E. coli* auxotroph occurs as the DAH form.[8] This is particularly convenient for integration with the chemical synthesis of DAH phosphonate. Refluxing the purified DAH in acidic methanol and recrystallization provide intermediate **4**. Thus conversion of the product of whole cell synthesis into a useful synthetic form obviates the need for phosphate monoester hydrolysis demanded by the immobilized enzyme synthesis. The difference in concentration of heptulosonate produced in whole cell relative to immobilized enzyme synthesis is not a serious problem, as large-scale operation of the whole cell synthesis is not difficult.[25b] This is due to the vigorous growth and general hardiness of *E. coli*.

When DAH is derived from immobilized phosphatase hydrolysis of crude enzyme reactor supernatant, a plethora of contaminants are present. Care must be exercised during the subsequent purification of the DAH by anion-exchange chromatography. Although the DAHP can be easily purified prior to hydrolysis of the phosphate monoester, such a strategem would effectively double the effort necessary to derive intermediate **4** from immobilized enzyme synthesis.

The diverse array of contaminants found in the immobilized enzyme system are also generated during the whole cell synthesis. An important difference is that for the whole cell synthesis the contaminants are restricted to the confines of the cell while DAH is excreted into the supernatant. The cells therefore function as trash bags filled with unwanted proteinaceous and organic contaminants. Their removal at the end of DAH accumulation by centrifugation is a significant purification step. The only major contaminants which remain in the solution are inorganic salts and D-glucose.

To remove the inorganic salts, the entire accumulation solution is acidified by filtration through a cation-exchange resin. Neutralization with lithium hydroxide generates the lithium salt of DAH as well as the lithium salts of the inorganics of the accumulation mixture. Due to the favorable solubility of the lithium salt of DAH in both methanol and water, addition of methanol to the solution precipitates the inorganics while DAH is left in solution. After removal of the methanol, an ion-exchange column affords a trivial separation of DAH from the D-glucose.

Other advantages attendant with integration of whole cell synthesis with the chemical synthesis of Scheme II are evident when the enzymes, cofactors, and cosubstrates demanded in immobilized enzyme synthesis are considered. With whole cell synthesis the researcher need not bother with enzyme purification and immobilization or the expense of purchasing the needed enzymes from commercial sources. Furthermore, whole cell synthesis does not require addition of the various cofactors and cosubstrates required by immobilized enzyme synthesis. DAHP synthetase, transketolase, pyruvate kinase, and hexokinase of *E. coli* are bathed in the cofactors and cosubstrates generated in situ by the intact, whole cell. The only additives required in the whole cell synthesis are the D-glucose and inorganic salts of the accumulation solution and the milligram amounts of aromatic amino acids necessary to correct for the organism's auxotrophy.

In the final analysis, combination of whole cell synthesis with chemical synthesis is the best option for swift derivation of DAH phosphonate from a simple, inexpensive carbohydrate. Immobilized enzyme synthesis is clearly the best alternative if the actual shikimate-pathway intermediate, DAHP, is desired. Such an approach also appears promising for obtaining radiolabeled DAHP. Thus while whole cell synthesis can expedite construction of DHQ synthetase targeted agents, immobilized enzyme synthesis can provide the molecular tools necessary for understanding the enzymology associated with plant DHQ synthetase.

Future attention need not be restricted to analogues of DAH. Enzymes of the shikimate pathway are overproduced in various strains of microbes.[22,26] Such enzymes can be immobilized and added to the immobilized enzyme system of Scheme III to allow ready access to a variety of shikimate-pathway metabolites. The same is true for whole cell synthesis. For each of the enzymes of aromatic amino acid biosynthesis, there exists a mutant strain of microbe whose auxotrophy results from absence of that particular enzyme. Either of these two approaches can enable the researcher to amass intermediates of aromatic amino acid biosynthesis. With short synthetic modification of these metabolites, the potential number of enzyme-targeted agents which can be constructed from either D-glucose or D-fructose is virtually unlimited.

Experimental Section

General. ^{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 $CDCl_3$ or CD_3CN 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 internal acetonitrile (CH_3CN, δ 3.69). 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. UV spectra were measured on a Hewlett-Packard 8450a UV–vis spectrometer. Melting points were uncorrected. Elemental analyses were performed by Micanal (Tucson, AZ). A Waters Associates ALC/510/R401/6000 system fitted with a μBondpak C_{18} column was utilized for high-pressure liquid chromatography (HPLC). Flash chromatogra-

(23) (a) Sprinson, D. B.; Rothschild, J.; Sprecher, M. *J. Biol. Chem.* **1963**, *238*, 3170. (b) Trigalo, F.; Level, M.; Szabo, L. *J. Chem. Soc., Perkin Trans. 1* **1975**, 600.

(24) Haslam, E. *The Shikimate Pathway*; Wiley: New York, 1974; Chapter 1, p 3.

(25) (a) The concentration of DAH in whole cell synthesis is generally 1 mM while that of DAHP produced in the immobilized enzyme synthesis is typically 10 mM. (b) Immobilized enzyme synthesis involves working with about 300 mL of crude DAHP-containing supernatant. Whole cell synthesis is optimized for purifying DAH from 4.5 L of growth solution.

(26) (a) Frost, J. W.; Bender, J. L.; Kadonaga, J. T.; Knowles, J. R. *Biochemistry* **1984**, *23*, 4470. (b) Rogers, S. G.; Brand, L. A.; Holder, S. B.; Sharp, E. S.; Bisckin, M. J. *Appl. Enviror. Microbiol.* **1983**, *46*, 37.

phy 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. AG 1-X8 was purchased from Bio-Rad, and Dowex 50 (H^+, 100–200 mesh) was purchased from Sigma. All chemicals were obtained from Aldrich and used without purification unless noted. Dimethylformamide was distilled from magnesium sulfate onto Linde 4A molecular sieves under nitrogen. Pyridine was distilled from calcium hydride under nitrogen. *N*-Bromosuccinimide was recrystallized from water. DAH, DAHP, and DAH phosphonate were assayed after periodate oxidation by reaction with thiobarbituric acid.[27]

Methyl (Methyl 4,5-di-*O*-acetyl-7-bromo-3-deoxy-D-*arabino*-heptulopyranosid)onate (11). Methyl (methyl 3-deoxy-D-*arabino* heptulopyranosid)onate (**4**) (1.50 g, 6.35 mmol) was dissolved in 60 mL of dimethylformamide with *N*-bromosuccinimide (2.26 g, 12.7 mmol) and cooled in an ice bath under a nitrogen stream. Triphenylphosphine (3.33 g, 12.7 mmol) was added over the course of 20 min. The reaction was heated at 50 °C for 2 h and cooled, and 15 mL of methanol was added. After the mixture 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 (methyl 3-deoxy-7-bromo-D-*arabino*-heptulopyranosid)onate. Treatment of this mixture with 10 mL of acetic anhydride in 40 mL of pyridine (18 h, room temperature) was followed by extraction from ethyl acetate with saturated copper sulfate, water (twice), saturated sodium bicarbonate (twice), water, and saturated sodium chloride. The organic layer was dried ($MgSO_4$), concentrated under reduced pressure, and subjected to flash chromatography (hexane/ethyl acetate, 2:1, v/v) to yield 1.41 g (3.68 mmol, 58%) of product **11** as a pale-yellow oil: IR (neat, NaCl) 3000–2840, 1750, 1438, 1370, 1236. 1160 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.81 (dd, J = 12, 12 Hz, 1 H), 1.94 (s, 3 H), 2.01 (s, 3 H), 2.43 (dd, J = 5, 8 Hz, 1 H), 3.27 (s, 3 H), 3.37–3.56 (m, 2 H), 3.76 (s, 3 H), 3.83–3.86 (m, 1 H), 4.86 (dd, J = 10, 10 Hz, 1 H), 5.24–5.26 (m, 1 H); ^{13}C NMR ($CDCl_3$) δ 20.6, 20.7, 31.0, 37.0, 51.2, 52.7, 68.6, 71.3 (2 C), 98.3, 167.3, 169.7, 169.8; MS, *m/e* (relative intensity) 325 (55), 323 (53), 233 (11), 231 (11), 205 (98), 203 (100). Anal. ($C_{13}H_{19}BrO_8$) C, H.

Ethyl (Methyl 4,5-di-*O*-acetyl-3-deoxy-D-*arabino*-heptulopyranosid)onate 7-(Diethyl phosphonate) (12). Pyranoside **11** (1.18 g, 3.08 mmol) was taken up in 11 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 1.16 g (2.56 mmol, 83%) of product **12** as a pale-yellow oil: IR (neat, NaCl) 3000–2900, 1744, 1638, 1450, 1372, 1238, 1166, 1043 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.22–1.28 (m, 9 H), 1.74 (dd, J = 12, 12 Hz, 1 H), 1.93 (s, 3 H), 2.00 (s, 3 H), 1.92–2.06 (m, 2 H), 2.46 (dd, J = 5, 10 Hz, 1 H), 3.32 (s, 3 H), 4.00–4.21 (m, 7 H), 4.76 (dd, J = 10, 10 Hz, 1 H), 5.22–5.27 (m, 1 H); ^{13}C NMR ($CDCl_3$) δ 14.0, 16.1 (J_{POCC} = 6 Hz), 16.2 (J_{POCC} = 6 Hz), 20.6, 20.7, 28.1 (J_{PC} = 144 Hz), 37.0, 51.2, 61.2 (J_{POC} = 6 Hz), 61.7, 61.9 (J_{POC} = 6 Hz), 67.0 (J_{PCCC} = 6 Hz), 68.6, 72.6, (J_{PCC} = 18 Hz), 98.2, 166.8, 169.7, 169.9; MS, *m/e* (relative intensity) 381 (29), 321 (13), 261 (100), 247 (20), 181 (51). Anal. ($C_{18}H_{31}O_{11}P$) C, H.

Ethyl (Methyl 4,5-di-*O*-acetyl-3-deoxy-D-*arabino*-heptulopyranosid)onate 7-Phosphonate (13). Fully protected phosphonate **12** (1.54 g, 3.39 mmol) was cooled to 0 °C under nitrogen and bromotrimethylsilane (3.13 g, 20.4 mmol) added dropwise. After the mixture stirred for 1 h at room temperature, excess reagent was removed under reduced pressure and the silyl ester hydrolyzed by vigorous stirring with 20 mL water for 30 min. The white solid was filtered, washed with dichloromethane, and then dried under vacuum to yield 1.02 g (2.57 mmol, 75%) of product **13** as a white powder. Material for combustion analysis was crystallized from water/acetonitrile (90:10, v/v) with standing at room temperature: IR (neat, NaCl) 3050–2850, 3000–2895, 1742, 1735, 1364, 1238, 1161, 1035 cm^{-1}; ^1H NMR (CD_3CN) δ 0.97 (t, J = 7 Hz, 3 H), 1.56 (dd, J = 12, 12 Hz, 1 H), 1.67 (s, 3 H), 1.74 (s, 3 H), 1.61–1.79 (m, 1 H), 1.87–1.98 (m, 1 H), 2.13 (dd, J = 5, 8 Hz, 1 H), 3.00 (s, 3 H), 3.70–3.80 (m, 1 H), 3.94–4.02 (m, 2 H), 4.58 (dd, J = 10, 10 Hz, 1 H), 4.84–4.92 (m, 1 H); ^{13}C NMR (CD_3CN) δ 14.3, 21.0 (2 C), 30.3 (J_{PC} = 144 Hz), 37.4, 52.0, 63.4, 68.2, 69.2 (J_{PCCC} = 6 Hz), 73.2 (J_{PCC} = 14 Hz), 99.1, 168.6, 170.9, 171.0. Anal. ($C_{14}H_{23}O_{11}P$) C, H.

(Methyl 3-Deoxy-D-*arabino*-heptulopyranosid)onic Acid 7-Phosphonate (14). Phosphonate **13** (0.60 g, 1.51 mmol) and 60.3 mL of 0.50 N aqueous sodium hydroxide were cooled separately in ice baths. The sodium hydroxide solution was added to **13** and stirred at 4 °C for 5 min. Dowex 50 (H^+ form, 25 mL), also cooled to 4 °C, was quickly added, making the solution pH <2.0. If more than 5 min is to elapse after addition of the NaOH solution, the reaction should be frozen in liquid nitrogen and the 25 mL of Dowex 50 (H^+ form) swirled in during melting. The entire mixture was loaded onto an additional 50 mL of Dowex 50 (H^+ form), and product **13** eluted with 225 mL of water at 4 °C. The eluant was concentrated under reduced pressure, the temperature being kept below 35 °C, to yield 0.41 g (1.45 mmol, 96%) of **13** as a hygroscopic white foam: IR (neat, NaCl) 3639–3000, 2968–2825, 1730, 1649, 1450, 1221, 1164, 1059 cm^{-1}; ^1H NMR (D_2O) δ 1.77 (dd, J = 12, 12 Hz, 1 H), 2.07–2.21 (m, 1 H), 2.35 (dd, J = 5, 8 Hz, 1 H), 2.42–2.54 (m, 1 H), 3.25 (dd, J = 10, 10 Hz, 1 H), 3.35 (s, 3 H), 3.78–3.97 (m, 2 H); ^{13}C NMR (D_2O) δ 31.7 (J = 144 Hz), 41.9, 53.9, 70.5 (J_{PCCC} = 6 Hz), 72.4, 77.3 (J_{PCC} = 16 Hz), 101.6, 174.5.

3-Deoxy-D-*arabino*-heptulosonic Acid 7-Phosphonate (1). Phosphonate **14** (0.66 g, 2.31 mmol) was dissolved in 46.2 mL of 25.0 mM HCl and the mixture stirred at 50–55 °C. After 9 days the reaction was 95–98% complete by ^1H NMR. After cooling, the solution was adjusted to pH 4.75 with sodium hydroxide, and the mixture was loaded onto 150 mL of AG 1-X8 equilibrated with 0.20 N sodium acetate at 4 °C. Washing with 0.20 L of 0.20 N sodium acetate was followed by eluting the phosphonic acid **1** with a linear gradient (0.70 + 0.70 L, 0.20–2.0 N) of sodium acetate, pH 4.75. The fractions were assayed, and those containing phosphonic acid were pooled, passed down 0.3 L of Dowex 50 (H^+), and then eluted with 0.90 L of water. The water was removed at reduced pressures below 30 °C to yield 0.56 g (2.08 mmol, 90%) of **1** as a hygroscopic white foam: IR (neat, NaCl) 3675–3028, 3000–2921, 1742, 1659, 1447, 1401, 1274, 1144, 1068 cm^{-1}; ^1H NMR (D_2O) δ 1.87 (dd, J = 12, 12 Hz, 1 H), 2.03–2.11 (m, 1 H), 2.27 (dd, J = 5, 8 Hz, 1 H), 2.38–2.46 (m, 1 H), 3.26 (t, J = 9 Hz, 1 H), 3.91–3.98 (m, 1 H), 4.03–4.11 (m, 1 H); ^{13}C NMR (D_2O) δ 32.1 (J_{PC} = 133 Hz), 41.4, 70.8 (J_{PCCC} = 9 Hz), 72.3, 77.8 (J_{PCC} = 13 Hz), 97.9, 175.6.

Enzyme Immobilization. Hexokinase (from Bakers yeast, immobilized on agarose), pyruvate kinase (from rabbit muscle, immobilized on polyacrylamide), transketolase, and alkaline phosphatase from unweaned calf intestinal mucosa were obtained from Sigma Chemical Co. DAHP synthetase was purified by the method of Schoner and Herrmann.[17] Transketolase, DAHP synthetase, and alkaline phosphatase were immobilized by the method of Whitesides.[28] The buffer used during transketolase immobilization was 0.3 M MOPS, pH 7.5, 0.01 M magnesium chloride, and 0.025 M D-fructose 6-phosphate. The ketose phosphate was necessary to protect the active site during immobilization. A unit yield of 60% (units assayed in enzyme-containing gel/units assayed in solution before immobilization) was achieved during immobilization of transketolase. The immobilized transketolase gel suspension was washed with ammonium sulfate solution followed by water and stored at 4 °C in distilled water. DAHP synthetase was immobilized in 0.3 M MOPS pH 7.7 with 0.05 M phosphoenolpyruvate to protect the enzyme active site. A unit yield of 20% was obtained during immobilization. The gel was washed and stored at 4 °C in distilled water. Substantial loss of activity was observed after 2 weeks of storage under these conditions. Neither the presence of dithiothreitol nor an argon atmosphere had any discernible impact on immobilized DAHP synthetase stability. As routine practice, immobilized DAHP synthetase was used within 1 week of immobilization of the enzyme.

Immobilized Enzyme Synthesis of DAHP. A typical immobilized enzyme synthesis utilized 200 mL (6 units) of immobilized DAHP synthetase, 80 mL (12 units) of immobilized transketolase, 0.6 g (8 units) of dried, immobilized pyruvate kinase, and 0.8 mL (15 units) of immobilized hexokinase. Production of DAHP was initiated by mixing the immobilized gel suspension with 200 mL of a solution containing D-fructose (2.7 mmol), D-ribose 5-phosphate (4.0 mmol), adenosine triphosphate (0.4 mmol), thiamin pyrophosphate (0.01 mmol), $MgCl_2 \cdot 6H_2O$ (2.0 mmol), phosphoenolpyruvate (6.0 mmol), and NaN_3 (0.2% to prevent microbial growth). The suspension was gently stirred at room temperature for 21 h, leading to the formation of 2.3 mmol (85%) of DAHP. At 21 h, D-fructose (2.7 mmol), D-ribose 5-phosphate (2.7 mmol), and PEP (5.4 mmol) were added to 50 mL of reactor supernatant. The pH of this solution was adjusted to pH 7.1 and the solution returned to the reactor. The suspension was stirred until D-fructose was exhausted, leading to 2.3 mmol of additional DAHP (85%).

At the end of the reaction immobilized enzymes were removed by centrifugation and stored in distilled water at 4 °C. Product DAHP in the supernatant was purified after filtration through Celite by anion-exchange chromatography at 4 °C. A volume of supernatant equivalent to 0.16 mmol of DAHP was loaded onto 80 mL of AG 1-X8 equilibrated with 0.25 M triethylammonium bicarbonate at pH 7.2. DAHP was

(27) Gollub, E.; Jalkin, H.; Sprinson, D. B. *Methods Enzymol.* **1971**, *17A*, 349.

(28) Pollak, A.; Blumenfeld, H.; Wax, M.; Baughn, R. L.; Whitesides, G. M. *J. Am. Chem. Soc.* **1980**, *102*, 6324.

eluted with a linear gradient (400 + 400 mL, 0.25–1.0 M) of triethylammonium bicarbonate, pH 7.2. Fractions containing DAHP were pooled and passed down 350 mL of Dowex 50 (H^+). The resulting DAHP solution after degassing was neutralized with lithium hydroxide, evaporated to dryness, and stored at −20 °C. Purified DAHP was identical by ^1H and ^{13}C NMR with authentic material and was a substrate for the DAHP specific enzyme, dehydroquinate (DHQ) synthetase.[8,26a]

Conversion of Immobilized Enzyme Synthesis Produced DAHP to 3-Deoxy-D-*arabino*-heptulosonic Acid (DAH, 3). Supernatant (300 mL) from an enzyme reactor containing 0.90 g (3.12 mmol) of DAHP was made 10 mM in magnesium chloride and 1 mM in zinc chloride via addition of the appropriate amounts of inorganic salts. Immobilized alkaline phosphatase was added to the solution and stirred at 37 °C for 72 h. The pH of the solution, initially 7.3, was readjusted to this value at 12-h intervals. Reaction progress was monitored by TLC (2:1:1 1-butanol/water/acetic acid) and by the disappearance of DAHP as determined by DHQ synthetase assay.[26a] Upon completion, the gel was removed by centrifugation (3000 g for 10 min) and washed 4 times with 100 mL of water. The combined supernatant and washes were passed through Celite and loaded onto 400 mL of AG 1-X8 equilibrated with 50 mM sodium acetate, pH 5.0. The column was washed with 0.50 L of 50 mM sodium acetate, pH 5.0, and DAH eluted with a linear gradient (2.0 + 2.0 L, 50–400 mM) of sodium acetate, pH 5.0. Those fractions containing DAH were combined, passed down 0.5 L of Dowex 50 (H^+) at 4 °C, and eluted with 1.5 L of water. Concentration under vacuum yielded 0.50 g of DAH (2.40 mmol, 77%) as a hygroscopic white foam: ^1H NMR (D_2O) δ 1.83 (dd, J = 12, 12 Hz, 1 H), 2.28 (dd, J = 5, 8 Hz, 1 H), 3.43 (dd, J = 10, 10 Hz, 1 H), 3.79–3.86 (m, 3 H), 3.94–3.99 (m, 1 H); ^{13}C NMR (D_2O) δ 41.3, 63.3, 71.1, 73.3, 76.7, 97.8, 175.7.

Whole Cell Synthesis of 3-Deoxy-D-*arabino*-heptulosonic Acid (DAH, 3). A cell suspension (4.5 L) of *E. coli* JB-5 (obtained by using protocol II of Frost and Knowles[8]) was centrifuged at 14 000 G for 10 min and the clear-yellow supernatant passed through a column (1 L) of Dowex 50 (H^+) at 4 °C. After being concentrated on a rotary evaporator (keeping the temperature below 35 °C), the solution was adjusted to pH 8.0 with freshly prepared lithium hydroxide, and methanol (1 L) was added with vigorous stirring at 4 °C for 1 h. The precipitate which formed was removed by filtration and the resulting filtrate concentrated under reduced pressure. The residue was then taken up in 200 mL of distilled, deionized water and adjusted to pH 5.0. The solution was loaded on a column (0.50 L) of AG 1-X8 equilibrated with 0.10 N sodium acetate, pH 5.0. The column was washed with 0.50 L of 0.10 N sodium acetate, and DAH was eluted with a linear gradient (2.0 + 2.0 L, 0.10–0.60 N) of sodium acetate, pH 5.0. Column fractions were assayed, and those containing DAH were combined, passed down 0.5 L of Dowex 50 (H^+) at 4 °C, and eluted with 1.5 L of water. The solution was concentrated to yield 1.47 g (7.05 mmol) of DAH as a hygroscopic, white foam.

Conversion of DAH (3) into Methyl (Methyl 3-deoxy-D-*arabino*-heptulopyranosid)onate (4). DAH (3) (1.47 g, 7.05 mmol) was azeotroped 3 times with toluene, immediately dissolved in 60 mL of methanol which was 0.75 N in HCl, and then refluxed for 24 h. After cooling, the solution was neutralized with solid lead carbonate $(PbCO_3)_2{\cdot}Pb(OH)_2$. Excess lead carbonate was removed by filtration through Celite, and activated charcoal was added to the filtrate. The mixture was allowed to stand at room temperature for 1 h and filtered again through Celite. The filtrate was concentrated under reduced pressure to ~5 mL, and **4** crystallized upon standing at room temperature. The fine white needles were filtered and washed with dichloromethane, and a second crop was collected by storing the filtrate at −20 °C for a total yield of 0.95 g (4.02 mmol, 57%): IR (NaCl, neat) 3453, 3370–3280, 2964–2800, 1736, 1460, 1440, 1341, 1281, 1191, 1121, 1081 cm^{-1}; ^1H NMR (D_2O) δ 1.76 (dd, J = 12, 12 Hz, 1 H), 2.36 (dd, J = 5, 8 Hz, 1 H), 3.25 (s, 3 H), 3.42 (dd, J = 10, 10 Hz, 1 H), 3.57–3.63 (m, 1 H), 3.75–4.00 (m, 3 H), 3.87 (s, 3 H); MS, *m/e* (relative intensity) 177 (100), 141 (17), 129 (32), 127 (41), 117 (43); mp 138 °C, with decomposition at 145 °C. Anal. ($C_9H_{16}O_7$) C, H.

Acknowledgment. We thank Prof. K. M. Herrmann for permission to use HE 102 (pKB 45). Work was supported by a grant-in-aid from Chevron Corporation and a Du Pont Young Faculty Grant.

Registry No. 1, 105103-72-8; **2**, 2627-73-8; **3**, 56742-43-9; **4**, 85549-51-5; **5**, 57-48-7; **6**, 50-99-7; **7**, 154-17-6; **8**, 91294-63-2; **9**, 91294-64-3; **10**, 91294-65-4; **11**, 105103-73-9; **11** (unacetylated), 105103-77-3; **12**, 105103-74-0; **13**, 105103-75-1; **14**, 105103-76-2; PEP, 138-08-9; DHQ synthase, 37211-77-1; DAHP synthase, 9026-94-2; $BrSiMe_3$, 2857-97-8; $HS(CH_2)_3SH$, 109-80-8; hexokinase, 9001-51-8; transketolase, 9014-48-6; pyruvate kinase, 9001-59-6; alkaline phosphatase, 9001-78-9; triethyl phosphite, 122-52-1; D-fructose-6-phosphate, 643-13-0; D-erythrose-4-phosphate, 585-18-2.

General Transannulation Approach to Angular Triquinanes. Total Syntheses of (±)-Pentalenene and (±)-*epi*-Pentalenene

Goverdhan Mehta* and Kasibhatla Srinivas Rao

Contribution from the School of Chemistry, University of Hyderabad, Hyderabad 500 134, India.
Received May 19, 1986

Abstract: A short, general approach for the synthesis of angular triquinanes is delineated. The key element of this approach is the carbonium-ion-mediated transannulation in the bicyclo[6.3.0]undecane system. New syntheses of appropriately functionalized bicyclo[6.3.0]undecanes **11a** and **11b** from cheap, abundantly available 1,5-cyclooctadiene have been developed. These have been cyclized in formic acid and elaborated to tricyclo[6.3.0.$0^{4,8}$]undecane derivatives **23** and **28**, respectively. These observations have been further extended to stereoselective syntheses of sesquiterpene hydrocarbon (±)-pentalenene (**6**) and (±)-*epi*-pentalenene (**10**) from commercial 1,5-dimethyl-1,5-cyclooctadiene (**29**). Site-selective cyclopentannulation of **29** provided access to the bicyclo[6.3.0]undecane-based enones **35** and **36** which underwent facile and stereospecific cyclization to tricyclic C_{13}-ketones **37** and **39**, respectively. A three-step protocol transformed them to (±)-pentalenene (**6**) and its C_9-epimer **10**, respectively.

While the presence of the tricyclo[6.3.0.$0^{4,8}$]undecane (**1**, angular triquinane) moiety was first recognized as a part structure in the pentacyclic sesterterpene retigeranic acid (**2**)[1] in 1972 and subsequently in the novel diterpenoids laurenene (**4**)[2] and crinipellin (**3**),[3] it is among the sesquiterpenoids that the skeleta based on **1** are most abundant and diverse. Up to date, five different C_{15}-angular triquinane carbon frameworks represented here by isocomene (**5**),[4,5] pentalenene (**6**),[6] silphinene (**7**),[7] silphiperfolene

(1) Kaneda, M.; Takahashi, R.; Litake, Y.; Shibata, S. *Tetrahedron Lett.* **1972**, 4609.
(2) (a) Corbett, R. E.; Lauren, D. R.; Weavers, R. T. *J. Chem. Soc., Perkin Trans. 1* **1979**, 1774. (b) Corbett, R. E.; Couldwell, C. M.; Lauren, D. R.; Weavers, R. T. *J. Chem. Soc., Perkin Trans. 1* **1979**, 1791.
(3) Anke, T.; Heim, J.; Knoch, F.; Mocek, U.; Steffan, B.; Steglich, W. *Angew. Chem.* **1985**, 709.
(4) (a) Zalkow, L. H.; Harris, R. N., III; Van Derveer, D.; Bertrand, J. A. *J. Chem. Soc., Chem. Commun.* **1977**, 452. (b) Zalkow, L. H.; Harris, R. N., III; Burke, N. I. *J. Nat. Prod.* **1979**, 42, 96.
(5) Bohlmann, F.; Le Van, N.; Pickhardt, J. *Chem. Ber.* **1977**, *110*, 3777.

0002-7863/86/1508-8015$01.50/0