was removed by repeated evaporation from water under reduced pressure. The product thus obtained was subjected to preparative electrophoresis in *p*H 5.5 pyridine-acetate buffer at 800 v for 7 hours.

The band exhibiting essentially no movement at this *p*H was further purified by electrophoresis in *p*H 3.6 acetic acid–sodium acetate buffer. In this manner it was possible to separate out a single ninhydrin-positive component which had the chromatographic properties of alanylglycine. The eluted substance was hydrolyzed in 6 N HCl and was found to contain only alanine and glycine. After one Edman degradation (Konigsberg and Hill, 1962), glycine was the only remaining ninhydrin-positive material.

Added in Proof

The amino acid sequence in ferrichrome A has been confirmed by crystallography (Zalkin, A., Forrester, J. D. and Templeton, D. H. [1964], *Science 146*, 261).

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Synthesis of 3,7-Dideoxy-D-*threo*-hepto-2,6-diulosonic Acid: A Study in 5-Dehydroquinic Acid Formation*

M. Adlersberg and D. B. Sprinson†

From the Department of Biochemistry, College of Physicians and Surgeons, Columbia University, New York City

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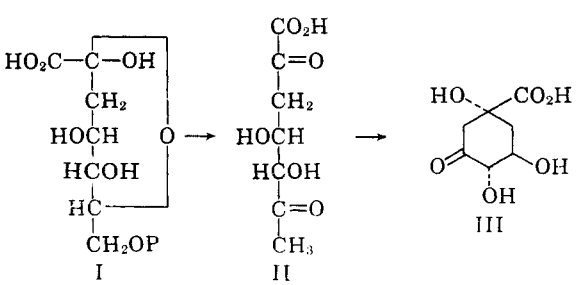
3,7-Dideoxy-D-*threo*-hepto-2,6-diulosonic acid (II), a postulated intermediate in the enzymic formation of 5-dehydroquinate, was synthesized from glucose via the intermediates V to XII. The key intermediate IX could also be obtained directly from compound VI or from VII. Although compound II was not converted to 5-dehydroquinate enzymically, it was cyclized to this compound by intramolecular aldol condensation at *p*H 11 or in imidazole buffer at *p*H 7, and pure 5-dehydroquinate was isolated. The "physiological conditions" under which compound II is cyclized spontaneously suggest that an enzyme-bound complex of compound II (perhaps in the form of the 6,7-enol) is active in the enzymic formation of 5-dehydroquinate from 3-deoxy-D-*arabino*-heptulosonate-7-phosphate (I).

Earlier investigations in this laboratory have shown that the cyclization reaction in the biosynthesis of the aromatic amino acids in *Escherichia coli* is the conversion of 3-deoxy-D-*arabino*-heptulosonic acid 7-phosphate (I) to 5-dehydroquinate (III) (Srinivasan *et al.*, 1963; Sprinson, 1960). The enzymic activity responsible for this conversion could not be fractionated into independent reactions, and evidence was presented to support the view that there were no free intermediates between substrate and product. These results were rationalized by a reaction scheme in which oxidation of compound I at C-5 facilitated elimination of orthophosphate, and reduction at C-5 then yielded 3,7-dideoxy-D-*threo*-hepto-2,6-diulosonic acid (II). The oxidation-reduction was assumed to be mediated by NAD, which was required for activity. An internal aldol condensation of compound II was postulated to yield III. Although the intermediates appeared to be enzyme-bound, it seemed worthwhile to synthesize compound II in order to test its chemical properties and its reactivity in the presence of 5-dehydroquinate synthetase.

The plan for the synthesis of compound II (see struc-



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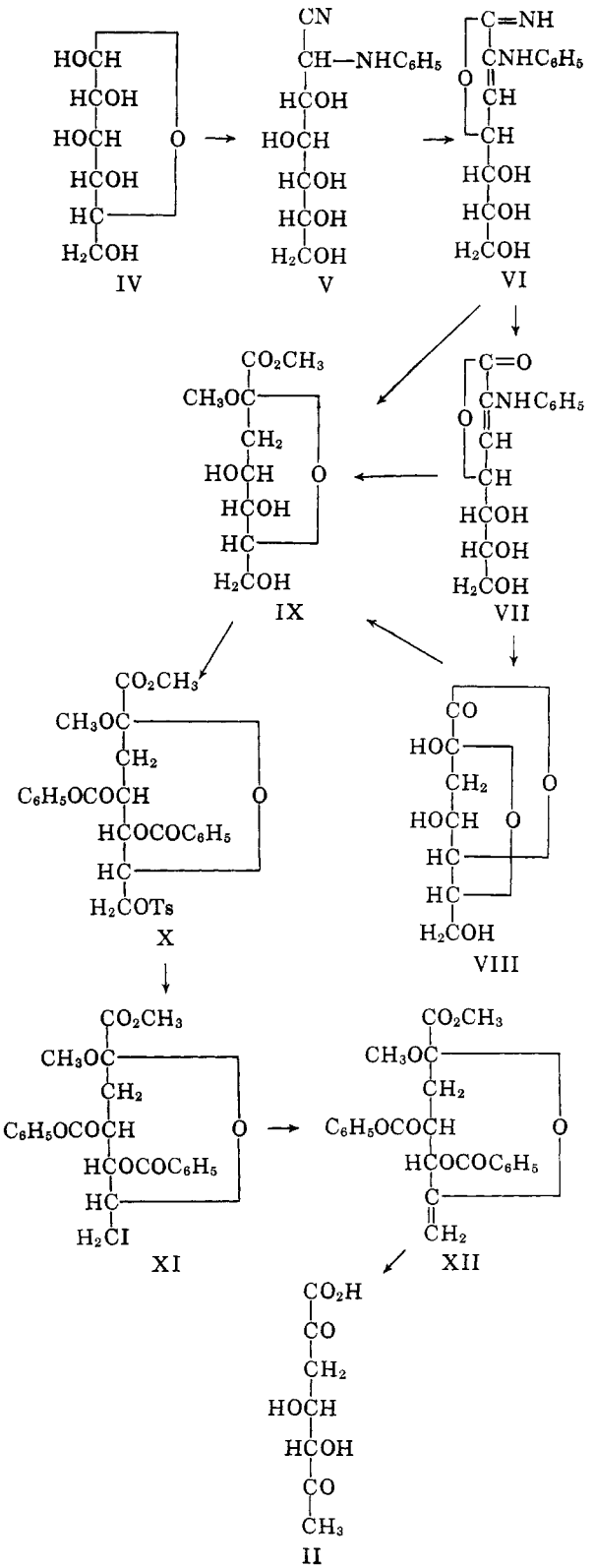
tures) was based on the formation of a glycoside of the 3-deoxy-D-*arabino*-heptulosonate ester (IX), and its conversion to the vinyl ether XII by methods so far applied only to aldoses (Helferich and Himmen, 1928). Removal of the protecting groups would then yield compound II.

Two routes are available to 3-deoxyulosonic acids and their derivatives. One route (MacGee and Doudoroff, 1954; Weissbach and Hurwitz, 1959; Sprinson *et al.*, 1963) consists of the conversion of 2-deoxyaldoses to 3-deoxyaldonic acids (via the cyanohydrins), and oxidation of the hydroxyl group on C-2 with a vanadium pentoxide reagent (Regna and Caldwell, 1944). The other route is based on the conversion of aldoses to the imidolactones of enamines (VI), and their stepwise hydrolysis to 3-deoxyulosonic acids (Kuhn *et al.*, 1959). The second route was chosen, since it avoided the large-scale chromatographic separation of 3-deoxyulosonic acid from unoxidized 3-deoxyaldonate (Sprinson *et al.*, 1963).

D-Glucose (IV) was converted to a 2-anilinoheptononitrile (V) by a modification of published procedures for the corresponding six carbon compounds (Kuhn *et al.*, 1959). The nitrile gave successively the imidolactone (VI), lactone (VII), and 3-deoxy-D-*arabino*-heptulosonate lactone (VIII),[1] which was readily converted by methanolic HCl to a methyl ester glycoside (IX).

In attempts to simplify this procedure it was found that the *enaminelactone* (VII) also reacted with anhydrous methanolic HCl to yield the same glycoside (IX). Furthermore, the *imidolactone* (VI) reacted similarly with methanolic HCl yielding the same product. The conversion of the iminoether structure of C-1 of VI to an ester is analogous to the conversion of iminoether hydrochlorides in methanol solution to esters with formation of dimethyl ether (McElvain and Stevens, 1947). A similar attack by the C-6 hydroxyl and methanol on the Schiff-base forms of compounds VI and VII would lead to displacement of anilinium ion and formation of the methylglycoside structure.

Tosylation of compound IX with one equivalent of tosyl chloride and then benzoylation gave the completely protected glycoside X, which on exchanging the tosyl group for iodide yielded compound XI. Dehydroiodination in the usual manner (Helferich and Himmen, 1928) gave the enol glycoside XII as a syrup with the expected elementary analysis and infrared spectrum.[2] The ester groups of compound XII were removed in alkaline solution; and, as would be expected from the enol glycoside structure of the resulting product, the *p*H of the decationized solutions, at room temperature, sufficed to hydrolyze the glycoside to 3,7-dideoxy-D-*threo*-hepto-2,6-diulosonic acid (II).



Solutions of compound II afforded a bis-2,4-dinitrophenylhydrazone in high yield, and showed the expected reactivity in the periodate-thiobarbiturate assay for ulosonic acids and semicarbazide assay for α-keto acids. With the latter reagent compound II showed two absorption maxima: one at 250 mμ, characteristic of α-keto acids, and one at 228 which is given by isolated carbonyl groups. The latter absorption maxi-

[1] The heptulosonate lactone (VIII) was obtained as a hygroscopic syrup which was converted in good yield to the known 2-hydroxyquinoxaline derivative (Sprinson *et al.*, 1963). The bicyclic structure of compound VIII was supported by its infrared spectrum, showing a band at 5.74 μ, which is consistent with a δ-lactone structure, and not with a carbonyl group at C-2. Evidence for this type of structure was also observed for compound I (the 7-phosphate of VIII) in acid solution (Sprinson *et al.*, 1963). After this investigation was completed, the synthesis of a crystalline enol lactone of compound VIII by the same series of reactions was reported in a brief communication (Paerels and Geluk, 1963). The syrup VIII had only weak bands at 6.04 and 6.13 μ, suggesting a small concentration of chelated enol and enol, respectively. However, a crystalline enol lactone was not obtained in this investigation.

[2] The dehydrohalogenation of compound XI by *AgF in pyridine* is in accord with the findings of Müller (1932) and of Helferich and Lang (1932) that vinyl ether formation occurs only when the halogen is neighboring to an oxygen bridge.

mum was observed also by treating 5-dehydroquinate with semicarbazide.

Attempts were made to convert compound II to 5-dehydroquinate by incubation with partially purified extracts of *E. coli*. Although these extracts were active in the conversion of 3-deoxy-D-*arabino*-heptulosonate-7-phosphate (I) to 5-dehydroquinate, there was no effect on compound II under various experimental conditions; compound II also did not inhibit the formation of 5-dehydroquinate from compound I. On the other hand, the base-catalyzed cyclization of compound II to 5-dehydroquinate could be readily demonstrated by paper chromatography, by enzymic conversion of the product to 5-dehydroshikimate, and by nutritional studies with aromatic auxotrophs. After treatment of compound II with alkali at *p*H 11, a product was formed which supported the growth of a mutant strain blocked *before* 5-dehydroquinate, but not of a strain blocked immediately *after* this compound, unless previously converted enzymically to 5-dehydroshikimate.

From a large-scale cyclization of compound II at *p*H 11 5-dehydroquinate was isolated in good yield, and shown to be identical with an authentic sample by melting point and infrared spectrum.

Only 70% of the diketone (II) which disappeared at alkaline *p*H was assayed as 5-dehydroquinate. As described under Experimental, it could be shown that this is owing in part to the instability of 5-dehydroquinate at alkaline *p*H (Weiss *et al.*, 1953). However, approximately 20% of compound II which disappeared remained unaccounted for. It is unknown whether this was due to the formation of the diastereomer of 5-dehydroquinate, or to more nonspecific side reactions.

The cyclization of compound II to 5-dehydroquinate occurred in unbuffered solutions even at neutral *p*H in the cold, and 50% yields of 5-dehydroquinate were obtained at 37° in imidazole buffer at *p*H 7, a catalyst used by Gutsche *et al.* (1962) to study the condensation of dihydroxyacetone with glyceraldehyde. The physiological conditions under which compound II undergoes intramolecular aldol condensation to form 5-dehydroquinate suggest a possible biological role for compound II in the enzymic conversion of I to 5-dehydroquinate. The reactive species in the internal aldol condensation of compound II would be the 6,7-enol, corresponding to the enolate anion of dihydroxyacetone phosphate which attacks the carbonyl carbon of glyceraldehyde-3-phosphate in the aldolase reaction. This enol may be bound to the enzyme through a sulfhydryl, or other group, prior to cyclization, and may not be available under ordinary conditions for exchange with external II in the keto form.

EXPERIMENTAL

*2-Anilino-2-deoxy-*D*-glycero-*D*-gulo(or ido)-heptononitrile* (V).—To a solution of 180 g (1.0 mole) of anhydrous glucose (IV) in 500 ml of dimethyl sulfoxide was added 100 ml (102 g, 1.1 moles) of freshly distilled aniline. The mixture was heated to 50° and kept at this temperature for 10 minutes, then chilled to 5°, treated with 100 ml (69 g, 2.5 moles) of cold anhydrous HCN, and allowed to stand overnight at room temperature. A stream of anhydrous N_2 was passed through the solution (kept at 35°) for 12–24 hours, unreacted HCN being absorbed in 50% NaOH. The solution was then cooled in an ice bath, and 800 ml of ice-cold water was added. After continued cooling for 1 hour the precipitated nitrile was removed by filtration, washed several times with cold water, and dried first in air, then *in vacuo* over P_2O_5, and finally in a vacuum oven (water pump) at 70°. Yield, 180 g (64%) of a white solid, mp 165–166° decomp. This material was used in the next step. An analytical sample was prepared by recrystallization from methanol, mp 165–166° decomp. $[\alpha]_D^{27}$ −132° (*c*, 1.12 in pyridine). Infrared bands (KBr): 4.47 (*w*; −C≡N), 6.24, 6.67 μ.

Anal. Calcd for $C_{13}H_{18}N_2O_5$ (282.3): C, 55.3; H, 6.38; N, 9.93. Found: C, 55.0; H, 6.65; N, 10.1.

*2-Anilino-2,3-dideoxy-*D*-arabino-hept-2-enonimido-1,4-lactone* (*VI*).—A stirred suspension of 200 g (0.71 mole) of the nitrile (V) in 1500 ml of absolute methanol was treated with a solution of 6.0 g (0.09 mole) of KOH in 1500 ml of absolute methanol, and heated with stirring at 45–50° until complete solution had taken place (15–20 minutes). The yellow solution was left overnight at room temperature, the methanol was removed by distillation *in vacuo* (bath temperature 30–35°), and the brown syrup which started to crystallize was triturated portionwise with 1500 ml of ice-cold water (until crystallization was complete). After chilling in an ice bath for 1 hour the yellow solid was removed by filtration, washed with cold water until the washings were neutral, and dried *in vacuo* first over KOH and then over P_2O_5. Yield 126 g (67%), mp 78° (occasionally 94–96°), which was used for the synthesis of compounds VII and IX. An analytical sample was prepared by recrystallization from ethyl acetate, mp 106–107°. (This compound is unstable at room temperature.) $[\alpha]_D^{27}$ −146° (*c*, 1.31 in methanol). Infrared bands (KBr): 5.95 (–O—C=NH), 6.01 (–C=CNHC$_6$H$_5$), 6.24, 6.50, 6.67, 6.92 μ. Ultraviolet spectrum (methanol): λmax 287 (ε 12,350), 236mμ (ε 11,100), shoulder.

Anal. Calcd for $C_{13}H_{16}N_2O_4$ (264.3): C, 59.1; H, 6.10; N, 10.6. Found: C, 59.0; H, 6.18; N, 10.6.

*2-Anilino-2,3-dideoxy-*D*-arabino-hept-2-enono-1,4-lactone* (*VII*).—A suspension of 61 g (0.23 mole) of the imidolactone (VI) in 610 ml of H_2O was heated on the steam bath for 50 minutes with occasional shaking. The resulting dark-brown mixture was filtered hot through a layer of glass wool and then on filter paper, and the filtrate was left overnight in the refrigerator. The brown crystalline product was crystallized from 300 ml of hot water with charcoal to yield 8.5 g of a light-brown solid, mp 152–155°, which on recrystallization from 160 ml of H_2O with charcoal yielded 6.8 g of pale-yellow crystals, mp 156–157°. A second crop of 0.75 g was obtained by concentrating the filtrate from the recrystallization; total yield 7.5 g (12.3%). An analytical sample was prepared by an additional recrystallization from water, mp 159–160°. $[\alpha]_D^{28}$ −227° (*c*, 0.72 in methanol). Infrared bands (KBr): 5.75 (–O–C=O), 6.00 (–C=C–NHC$_6$H$_5$), 6.24, 6.50, 6.67, 6.92 μ. Ultraviolet spectrum (absolute alcohol): λ_{max} 292 mμ (ε 13,200), 240 mμ (ε 8600).

Anal. Calcd for $C_{13}H_{15}NO_5$ (265.3): C, 58.9; H, 5.70; N, 5.28. Found: C, 58.9; H, 5.72; N, 5.29.

*3-Deoxy-*D*-arabino-heptulopyranosono-1,5-lactone* (*VIII*).—A suspension of 12 g (0.045 mole) of the enaminelactone (VII) and 108 ml of moist Dowex 50-X8 (H^+, 50–100 mesh) in 108 ml of H_2O was heated with magnetic stirring at 65–70° for 45 minutes. The resin was removed by filtration and washed with warm water, the combined filtrate and washings were concentrated *in vacuo*, and the resulting light-yellow syrup was twice dissolved in absolute ethanol and reconcentrated. An aqueous solution of the syrup was shaken with charcoal for 24 hours, and after removal of the charcoal by filtration the filtrate was concentrated *in vacuo*. The residue was repeatedly dissolved in absolute ethanol and evaporated to dryness, and was finally dried *in vacuo* over P_2O_5. Yield 8 g of a colorless hygroscopic syrup (which was free of nitrogen). $[\alpha]_D^{28}$

+33° (*c*, 1.78 in H_2O). Infrared bands (KBr)[3]: 5.74 (–O–C=O), 6.04 (*w;* chelated enol), 6.13 (*w;* C=C—OH) μ. The molar absorption of this product in the periodate-thiobarbiturate assay for ulosonic acids (Warren, 1959), and in the semicarbazide reaction (MacGee and Doudoroff, 1954) was the same as that of 3-deoxy-D-*arabino*-heptulosonate 7-phosphate (Sprinson *et al.*, 1963).

2-Hydroxyquinoxaline Derivative of Compound VIII.—A solution of 0.39 g (2.05 mmoles, assuming 100% purity) of the syrup (VIII) in 10 ml of H_2O was treated with 0.25 g. (2.3 mmoles) of *o*-phenylenediamine. The mixture was heated gently to bring the reagent into solution, and was allowed to stand overnight at room temperature. Yield of derivative 0.36 g (68%), mp 194–195°. After two recrystallizations from water, mp 197–198°; previously reported (Sprinson *et al.*, 1963) 195–196°. Ultraviolet spectrum (50% methanol), λ_{max} 337 mμ (ε 7600), 287 mμ (ε 6500).

*Methyl (Methyl 3-Deoxy-*D*-arabino-heptulopyranosid)onate (IX).*—METHOD A.—A solution of 6.23 g (0.033 mole) of the syrupy lactone (VIII) in 600 ml of 2% anhydrous HCl in methanol was allowed to stand overnight at room temperature, then refluxed gently for 6 hours, chilled in an ice bath, and neutralized with anhydrous Ag_2CO_3 (protection from moisture throughout). The precipitated AgCl was removed by filtration and washed several times with absolute methanol, and the combined filtrate and washings were evaporated *in vacuo* to a thick syrup which crystallized. Recrystallization from ethyl acetate gave 3.2 g (41%) of white crystals (mp 145–146°), the mother liquors of which yielded 0.4 g, mp 141° (overall yield from compound VI, 5%). An analytical sample was prepared by recrystallization from ethyl acetate, mp 148°. $[\alpha]_D^{28}$ +78.2° (*c*, 1.01 in methanol). Infrared band (KBr): 5.74 (–CO—OCH_3) μ.

Anal. Calcd for $C_9H_{16}O_7$ (236.2): C, 45.8; H, 6.83. Found: C, 46.1; H, 7.08.

METHOD B.—A solution of 7.2 g (0.027 mole) of the enaminelactone (VII) in 365 ml of 4% anhydrous HCl in methanol was treated exactly as described above for the methanolysis of compound VIII. After removal of AgCl, the cloudy filtrate (and washings) was clarified with charcoal, and the clear solution was stirred for 1 hour with 6 g of dry Dowex 50-X8 (H^+, 50–100 mesh) to remove aniline. The resin was removed by filtration and washed with absolute methanol, and the combined colorless filtrate and washings were concentrated *in vacuo* to a syrup which crystallized. Recrystallization from 200 ml of ethyl acetate gave 3.7 g. (58%), mp 144–146°; no depression in mixed mp with material obtained by method A (overall yield from compound VI, 7%).

METHOD C.—A solution of 52.9 g (0.2 mole) of the imidolactone (VI) in 500 ml of absolute methanol was treated with 500 ml of 8% methanolic HCl. After standing overnight at room temperature the reaction mixture was refluxed gently for 6 hours, chilled in an ice bath, and neutralized with lead carbonate. The precipitate was removed by filtration and washed several times with methanol, and the combined filtrate and washings were evaporated *in vacuo* to a thick, brown syrup which was dried over P_2O_5, and extracted twice with 300 ml of hot ethyl acetate. Insoluble material was removed by filtration, the filtrate (after treatment with charcoal) was chilled and seeded, and the crude ester was collected. Recrystallization from 400 ml of hot ethyl acetate with charcoal gave 3.8 g (8%) of pure material, mp 147–148°. The mother liquors from the first crystallization were concentrated *in vacuo* and the residual brown syrup was treated with Dowex 50 in methanol to remove aniline, and then with 4% methanolic HCl as before to yield 1.6 g of ester, mp 144–145°. Total yield 5.4 g (11%). (Method C was the method of choice.)

*Methyl (Methyl 4,5-Di-O-benzoyl-3-deoxy-7-O-tosyl-*D*-arabino-heptulopyranosid)onate (X).*—A stirred solution of 4.7 g (20 mmoles) of the methylglycoside (IX) in 80 ml of pyridine (dried over BaO) was chilled in an ice bath and treated portionwise over a period of 20 minutes with 4.2 g (22 mmoles) of *p*-toluenesulfonyl chloride. Stirring in the cold was continued for 1 hour, and the mixture was allowed to stand overnight at room temperature. The clear solution was chilled and treated similarly with 5.1 ml (6.2 g, 44 mmoles) of benzoyl chloride. The reaction mixture was then added with efficient stirring to 600 ml of water cooled to 0°, and the resulting suspension was stirred at ice-bath temperature for 5 hours. The white precipitate was removed by filtration, washed several times with cold water and once with a small volume of cold 50% methanol, and dried *in vacuo* over P_2O_5 (yield 11 g). Crystallization from 220 ml of methanol gave 9.0 g (75%), mp 144–146°. An analytical sample was prepared by recrystallization from methanol, mp 146–147°. $[\alpha]_D^{24}$ −30.4° (*c*, 1.39 in acetone). Infrared bands (KBr): 5.72 (–CO–OCH_3), 5.76 (–CO–OC_6H_5), 6.25, 6.70, 6.90, and corresponding to –O–SO_2–R 7.35, 8.51 μ.

Anal. Calcd for $C_{30}H_{30}O_{11}S$ (598.6): C, 60.2; H, 5.05; O, 29.4; S, 5.35. Found: C, 60.0; H, 4.95; O, 29.1; S, 5.60.

*Methyl (Methyl 4,5-Di-O-benzoyl-3,7-dideoxy-7-iodo-*D*-arabino-heptulopyranosid)onate (XI).*—A solution of 2.75 g (4.6 mmoles) of the tosylate (X) and 2.75 g of anhydrous NaI in 55 ml of dry acetone was heated in a sealed tube at 100–105° for 4 hours. Sodium *p*-toluenesulfonate was removed by filtration and washed thoroughly with dry acetone and the combined filtrate and washings were concentrated to dryness *in vacuo*. The residual solid was stirred in an ice bath with 50 ml of ice-cold water for 2 hours, and the insoluble material was removed by filtration, washed with cold water, and dried. The crude product (2.0 g) was dissolved in 10 ml of hot methanol and allowed to cool at room temperature and, after nearly complete crystallization, in the refrigerator. Yield, 1.6 g (63%) of white crystals, mp 105–106°. An analytical sample was prepared by recrystallization from methanol, mp 105–106°. $[\alpha]_D^{23.5}$ −22.8° (*c*, 2.53 in chloroform). Infrared bands (KBr): 5.75 (–CO–OCH_3), 5.80 (–CO–OC_6H_5), 6.25, 6.70, 6.90 μ.

Anal. Calcd for $C_{23}H_{23}O_8I$ (554.3): C, 49.8; H, 4.18; I, 22.9. Found: C, 49.5; H, 4.01; I, 22.8.

*Methyl (Methyl 4,5-Di-O-benzoyl-3,7-dideoxy-*D*-arabino-hept-6-enulopyranosid)onate (XII).*—Anhydrous AgF (3.3 g) was added to a solution of 1.67 g (3.0 mmoles) of iodo compound (VII) in 17 ml of pyridine (dried over BaO), and the mixture (protected from light) was shaken for 7 days at room temperature. The dark-brown reaction mixture was shaken with 50 ml of anhydrous ether, and the upper layer was removed by decantation. After repeating this extraction nine times the ethereal solution was concentrated *in vacuo*, and the residual brown syrup was extracted with chloroform. Insoluble material (mostly inorganic) was removed by filtration, and the filtrate was concentrated to a syrup which was treated twice more in a similar manner with methanol plus ether for removal of tenacious inorganic impurities. Solvents were re-

[3] The KBr plate was obtained by lyophilizing an aqueous solution of the syrup and KBr, and pressing the resulting powder.

moved by distillation *in vacuo*, and the residue was dried over P_2O_5 (oil pump), leaving 0.47 g (36%) of a yellow syrup which could not be crystallized. Infrared and ultraviolet spectra of this product were identical with those of the purified material reported here.

This product was further purified by partition chromatography on Celite using the solvent system isooctane-methanol-water (10:9:1). Acid-washed Celite (Johns-Manville No. 545) was washed on a column with methanol and ether, and dried *in vacuo* and then in an oven at 100°. The Celite (85 g) was mixed thoroughly with the stationary polar phase of the solvent system in a ratio of 0.75 ml of solvent to 1 g of Celite, and packed in 29 charges into a column 3 × 60 cm (Kelly *et al.*, 1962). The crude syrup (100 mg) was dissolved in 3.75 ml of the stationary phase and mixed with 5 g of Celite and 1 ml of the mobile phase, and the mixture was loaded in 2 charges on top of the column. Partition was carried out with the mobile phase, and fractions of 10 ml were collected on a fraction collector and monitored by measuring absorption at 274 mμ. The main component was obtained in fractions 22–29 (distribution coefficient, 4; predicted, 5), which were combined and evaporated to dryness at the water pump (bath temperature 35°). The residue was dissolved in methanol and evaporated to dryness, and this process was repeated. Drying *in vacuo* over P_2O_5 yielded a colorless glass (66 mg). $[\alpha]_D^{25}$ −36.3° (*c*, 1.56 in methanol). Infrared spectrum (KBr): bands at 5.70 (-CO-OCH_3), 5.78 (-CO-OC_6H_5), 6.24, 6.70, 6.88; and for $H_2C{=}C{-}O{-}$ (Meakins, 1953) 5.99, 12.45 μ. Ultraviolet spectrum (95% ethanol), λ_{max} 282 mμ (ε 1470), 274 mμ (ε 1800), 231 mμ (ε 26,700). For methyl benzoate, λ_{max} 280 mμ (ε 692), 273 mμ (ε 832), 228 mμ (ε 11,000).

Anal. Calcd for $C_{23}H_{22}O_8$ (426.4): C, 64.8; H, 5.20. Found: C, 65.1; H, 5.73.

3,7-Dideoxy-D-threo-hepto-2,6-diulosonic Acid (II).—To 1.2 ml of 1% NaOH in 90% ethanol (0.29 mmole of base) was added 27 mg (0.064 mmole) of chromatographically purified compound XII. The slightly yellow solution was allowed to stand for 3.5 hours at room temperature, and was then clarified by centrifugation and passed three times through a 1-ml column of Dowex 50-X8 (H^+, 50–100 mesh). The resin was washed thoroughly with water, and the combined eluates were concentrated to a small volume on the water pump (bath temperature 30°). After removal of benzoic acid by extraction with ether, the aqueous solution was freed of ether and concentrated by evaporation *in vacuo*, and diluted to 5 ml. The yield of compound II was 54 μmoles (85%) when determined under the strongly acidic conditions of the periodate-thiobarbiturate procedure (Warren, 1959), with a solution of the potassium salt of compound I as a standard. In the semicarbazide procedure at *p*H 5 (MacGee and Doudoroff, 1954) the yield was only 35 μmoles of α-keto acid on the freshly prepared solution, as measured against a sodium pyruvate standard. However, the yield increased to a maximum value of 50 μmoles after the solution was allowed to stand overnight in the refrigerator at its own *p*H (2.5). Such solutions appeared to be stable for at least 1 week at 0°.

Assuming the concentration of compound II as given by the periodate-thiobarbiturate procedure, the ultraviolet spectrum of compound II in semicarbazide solution at *p*H 5 was λ_{max} 250 mμ (ε 8700), 228 mμ (ε 14,800). With the latter reagent, 5-dehydroquinate gave λ_{max} 229 (ε 14,200).

Bis-(2,4-dinitrophenylhydrazone) of Compound II.—A solution (1 ml) of compound II, obtained as described above from 100 mg of compound XII (unchromatographed syrup), was treated with a solution of 93 mg (0.47 mmole) of 2,4-dinitrophenylhydrazine in 8.5 ml of 2 N HCl, and allowed to stand overnight in the refrigerator. The yellow precipitate was removed by filtration, washed with ice-cold 2 N HCl and water, and dried *in vacuo* over P_2O_5. Yield, 78 mg (57% assuming 100% purity for compound XII), mp 144–145° decomp. After crystallization from 95% ethanol–ethyl acetate, mp 145–148° decomp. An analytical sample was prepared by recrystallization from the same solvents, mp 148–151° decomp.

Anal. Calcd for $C_{19}H_{18}N_8O_{12}$ (550.4): C, 41.5; H, 3.29; O, 34.9; N, 20.4. Found: C, 41.0; H, 3.45; O, 34.4; N, 20.6.

Attempted Enzymic Conversion of Compound II to 5-Dehydroquinate.—Partially purified extracts from *E. coli* were used as source of enzyme under previously described conditions (Srinivasan *et al.*, 1963). No significant formation of 5-dehydroquinate from compound II could be observed, although compound I was converted nearly quantitatively to 5-dehydroquinate by these extracts. There was no inhibition of the conversion of compound I by incubation in the presence of compound II.

Cyclization of the Diketone (II) to 5-Dehydroquinate.—A solution of compound II (1 ml, 10.8 μmoles), prepared as previously described from purified compound XII, was diluted with 2 ml of water, brought to *p*H 11.0 with 0.01 N KOH, and diluted to a final volume of 5 ml. At zero time and after 1 hour at room temperature an appropriately diluted aliquot (up to 0.05 μmole) was assayed for ulosonic acid (Warren, 1959). The results showed that 6.9 μmoles of compound II remained after 1 hour, or that 3.9 μmoles of compound II were converted to compounds incapable of reacting in the test for ulosonic acids.

The above solution was assayed for 5-dehydroquinate by enzymic conversion to 5-dehydroshikimate (Mitsuhashi and Davis, 1954). An ammonium sulfate fraction (0.3–0.5 saturated) of *E. coli* 83–24 was used. It was found that 2.9 μmoles of 5-dehydroquinate were present, equivalent to 72% of compound II which disappeared. A control solution of 5-dehydroquinate (Haslam *et al.*, 1963) kept at *p*H 11 for 1 hour showed 92% remaining by enzymic assay.

The alkaline solutions of compound II were also assayed for 5-dehydroquinate by growth response for *Aerobacter aerogenes* mutant A170-143S1, an organism blocked before 5-dehydroquinate (Davis and Weiss, 1953; see Levin and Sprinson, 1964). The yield of 5-dehydroquinate was found to be the same as with the enzymic assay. When tested with *E. coli* mutant 83–1, which is blocked immediately after 5-dehydroquinate (Weiss *et al.*, 1953), there was no growth response. However, after incubation with 5-dehydroquinase (as discussed earlier), bioassay with *E. coli* 83-1 showed an amount of 5-dehydroshikimate equivalent to that found enzymically.

A solution of compound II, prepared as described, was adjusted to *p*H 7.5 and kept at 0° for 24 hours. The acid and neutral solutions of compound II and a solution of 5-dehydroquinate were spotted on Whatman No. 1 paper and chromatographed by the ascending technique in the solvent system benzyl alcohol–*t*-butyl alcohol–isopropyl alcohol–water (3:1:1:1) containing 2% of 90% formic acid (Haslam *et al.*, 1961). 5-Dehydroquinate, R_F 0.3, was detected by an aniline spray reagent (Yoshida and Hasegawa, 1957), and compound II, R_F 0.5, was detected by the periodate-thiobarbiturate reagents (Srinivasan and Sprinson, 1959). The acid solution of compound II showed the presence of diketone only, while the neutralized solution of com-

pound II showed a 5% conversion to 5-dehydroquinate.

Isolation of 5-Dehydroquinic Acid.—Compound XII was purified on a large scale by absorption chromatography on Celite. A column 2 × 22 cm was prepared from 15 g of absorbent (washed and dried as described before) by slurrying with ligroin. The crude syrup (1 g) in 3 ml of benzene was loaded on the column, and elution with 200 ml of benzene gave 900 mg of essentially pure material. Hydrolysis of 2.3 g of purified XII, and cyclization of compound II at *p*H 11 (final volume 100 ml), were carried as described above. The alkaline solution was treated with Dowex 50 (H^+), and the filtrate was adjusted to *p*H 8 with NH_4OH. 5-Dehydroquinate was isolated by gradient elution from Dowex 1 acetate (Haslam *et al.*, 1963). Crystallization from acetone-chloroform gave 170 mg, mp 129–131°. Recrystallization gave 120 mg of material, mp 134–136°, which did not depress the melting point of authentic 5-dehydroquinate, and was identical with it in infrared spectrum and ultraviolet absorption in semicarbazide solution.

Imidazole-catalyzed Cyclization of Compound II to 5-Dehydroquinate.—A solution of compound II (1 ml, 16 μmoles) was brought to *p*H 6.8 with dilute KOH, treated with 0.27 ml of 3 M imidazole hydrochloride buffer, *p*H 7 (final volume 1.3 ml; imidazole concentration 0.6 M), and kept at 37°. After 23 and 44 hours the 5-dehydroquinate determined enzymically, as described above, was 6.6 and 8.2 μmoles, representing 41 and 51% yields, respectively.

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