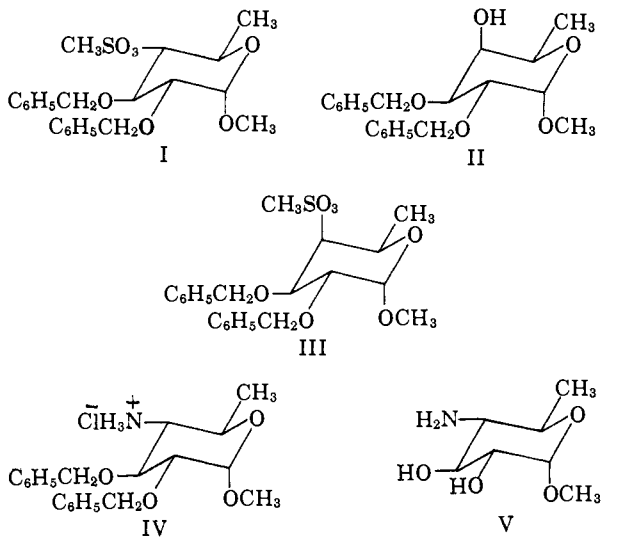
Synthesis of Viosamine (4-Amino-4,6-dideoxy-D-glucose) by Double Inversion at C-4 and Identification with the 4-Amino-4,6-dideoxyhexose from *Escherichia coli* Strain B

Sir:

In 1960 an unidentified sugar was found linked to thymidine diphosphate in extracts of *E. coli* strain B.[1] This nucleotide has more recently been synthesized enzymatically by extracts of this organism by a stereospecific transamination of TDP-4-keto-6-deoxy-D-glucose with L-glutamate and pyridoxal phosphate.[2]

Periodate oxidation of the sugar obtained from the thymidine nucleotide isolated from extracts of *E. coli* strain B resulted in the uptake of two moles of periodate with formation of two moles of formic acid (determined with formic acid dehydrogenase from a strain of *E. coli*).[3] By successive oxidation with periodate (pH 5.0) and bromine (pH 5.5) and deacetylation with 4 *N* HCl at 100° for 6 hr., the remaining four carbon atoms were converted to an amino acid identified as allothreonine. Since this amino acid did not serve as a substrate for L-threonine dehydrase (for which L-allothreonine is also a substrate), it must have the D-configuration. D-Allothreonine was also obtained from TDP-4-acetamido-4,6-dideoxyhexose synthesized enzymatically with enzymes from *E. coli* strain B thus establishing that the configuration at C-4 to C-6 was the same as that found in D-glucose. These degradative studies were thus compatible with the supposition that this sugar was 4-acetamido-4,6-dideoxy-D-glucose, although they provided no data from which the configurations at C-2 and C-3 could be deduced. In addition the n.m.r. spectrum in D_2O of the amino sugar hydrochloride isolated from the enzymatically synthesized nucleotide supported this structure, although it also did not distinguish possible configurations. This sugar could be distinguished chromatographically from a similar sugar 4-amino-4,6-dideoxy-D-galactose,[4] isolated from *E. coli* strain Y-10 and synthesized enzymatically by extracts of this organism. Contemporaneously with this work, Wheat, *et al.*,[5] isolated and partially characterized a crystalline 4-amino-4,6-dideoxyhexose from the lipopolysaccharide of *Chromobacterium violaceum*, later shown by Stevens, Wheat, *et al.*,[6] to be identical with synthetic 4-amino-4,6-dideoxy-D-glucose (viosamine). In this communication, the synthesis of viosamine by a double inversion procedure will be reported together with data which prove that the sugar from *E. coli* strain B is also 4-amino-4,6-dideoxy-D-glucose, and is, therefore, the 4-epimer of the sugar from *E. coli* strain Y-10.

Methyl 2,3-di-*O*-benzyl-6-deoxy-4-*O*-methylsulfonyl-α-D-glucopyranoside[4] (I) was treated with sodium benzoate in dimethylformamide followed by aqueous sodium hydroxide to give 67% yield of methyl 2,3-di-*O*-benzyl-6-deoxy-α-D-galactopyranoside (II), m.p. 82–83°, $[\alpha]^{23}D$ +48.5° (*c* 1.04, $CHCl_3$). Mesylation in pyridine gave 75% of methyl 2,3-di-*O*-benzyl-6-deoxy-4-*O*-methylsulfonyl-α-D-galactopyranoside (III), m.p. 73–74°, $[\alpha]$ +71.4° (*c* 1.05, $CHCl_3$). The mesyloxy group at C-4 in III was converted with inversion to an azido group by treatment with lithium azide in dimethylformamide and the azido group was then reduced with lithium aluminum hydride to give methyl 4-amino-2,3-di-*O*-benzyl-4,6-dideoxy-α-D-glucopyranoside hydrochloride (IV), m.p. 180–181°, $[\alpha]^{25}D$ +46.2 (*c* 1.1, H_2O), in 57% over-all yield from III. The benzyl ether groups were removed by hydrogenation to give 80% of methyl 4-amino-4,6-dideoxy-α-D-glucopyranoside (V), m.p. 116–117°, identical in all respects with "methyl viosamine."[6] Compound V was converted to the N-acetyl derivative; the latter was hydrolyzed in 78% yield to viosamine. A small amount of viosamine was converted to the N-acetyl derivative which traveled as a single spot with $R_{rhamnose}$ 0.89 in pyridine–ethyl acetate–water, 1.4:3.6:1.15 (system A), $R_{rhamnose}$ 1.02 in 1-butanol–ethanol–water, 13:8:4 (system B), and $R_{rhamnose}$ 0.97 in 1-butanol–pyridine–water, 6:4:3 (system C). Okazaki, *et al.*[7] reported $R_{rhamnose}$ 0.90 in system A, 1.03 in system B, and 0.99 in system C.



This sugar was also synthesized enzymatically with a partially purified preparation from *E. coli* strain B[2] employing a radioactive substrate, as described in the preceding communication.[4] The synthetic sample was added to a solution of the enzymatically synthesized radioactive compound. Upon crystallization, all of the radioactivity was obtained in the crystals and amounted to 214 counts per min. per mg. The counts remained constant through three additional recrystallizations and thus confirmed the structure of the sugar from *E. coli* strain B as 4-amino-4,6-dideoxy-D-glucose.

In order to demonstrate that an isomeric carbohydrate derivative could be removed by crystallization, a small sample of 4-amino-4,6-dideoxy-D-glucose was inoculated with radioactive 4-amino-4,6-dideoxy-D-galactose. Two recrystallizations removed 88% of the radioactivity.

(1) R. Okazaki, T. Okazaki, and Y. Kuriki, *Biochim. Biophys. Acta*, **38**, 384 (1960).

(2) M. Matsuhashi and D. N. Dietzler, *Federation Proc.*, **23**, 170 (1964); M. Matsuhashi and J. L. Strominger, *J. Biol. Chem.*, **239**, 2454 (1964).

(3) R. Okazaki, T. Okazaki, and S. Suzuki, Abstracts, 35th Annual Congress, Japanese Biochemical Society, 1962, p. 403.

(4) C. L. Stevens, P. Blumbergs, D. Otterbach, J. L. Strominger, M. Matsuhashi, and D. N. Dietzler, *J. Am. Chem. Soc.*, **86**, 2937 (1964).

(5) R. W. Wheat, E. L. Rollins, and J. M. Leatherwood, *Biochem. Biophys. Res. Commun.*, **9**, 120 (1962).

(6) C. L. Stevens, P. Blumbergs, F. A. Daniher, R. W. Wheat, A. Kiyomoto, and E. L. Rollins, *J. Am. Chem. Soc.*, **85**, 3061 (1963).

(7) T. Okazaki, R. Okazaki, J. L. Strominger, and S. Suzuki, *Biochem. Biophys. Res. Commun.*, **7**, 300 (1962).

Finally, the free amino sugars and their N-acetyl derivatives synthesized chemically or enzymatically with enzymes from *E. coli* strain B, or isolated from *C. violaceum*, had identical mobilities on paper chromatography in five solvent systems.

As in the case of *E. coli* strain Y-10, the large amount of TDP-4-acetamido-4,6-dideoxy-D-glucose found in *E. coli* strain B is presumably a reflection of the inability of this strain to utilize the nucleotide for polysaccharide synthesis. *E. coli* strain B and *C. violaceum* (which contains viosamine in its lipopolysaccharide) are unusual in containing 4-amino-4,6-dideoxy-D-glucose, since 14 other strains of enteric bacteria which have been examined are able to synthesize only the sugar with D-*galacto* configuration.

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Photochemical Isomerization of Di-*t*-butylbenzenes

Sir:

As an extension of our studies on the chemistry of *o*-di-*t*-butylbenzene,[1] we have examined the photoirradiation of this hydrocarbon. In contrast to the conversion of 1,2,4-tri-*t*-butylbenzene into a substituted bicyclo[2.2.0]hexadiene (Dewar-type "benzene") on irradiation in ether solution with a mercury arc lamp through a Vycor filter,[2] *o*-di-*t*-butylbenzene when similarly irradiated in an all-quartz apparatus[3] undergoes a new kind of isomerization to form a 1:4 photostationary equilibrium mixture of *m*- and *p*-di-*t*-butylbenzene.[4,4a]

Irradiations in our work were conducted in dilute ether solution, under nitrogen, with a 100-watt Hanovia Type 8A36 quartz-jacketed mercury light source placed in a water-cooled quartz immersion well. Product isomer ratios were estimated by characteristic absorption peaks in the infrared and/or n.m.r. spectra. Results of a typical run (510 mg. of *o*-di-*t*-butylbenzene in 1 l. of ether) are summarized in Table I, from which it is noted that the first stage of the isomerization was complete in about 70 hr. Likewise, starting from either *m*- or *p*-di-*t*-butylbenzene, the same photostationary composition was reached. Unfortunately, considerable amounts of unidentified by-products were also formed, and the total yield of mixed di-*t*-butylbenzenes after 75 hr. was found to be only about 20% as estimated by n.m.r. analysis.

TABLE I
PHOTOCHEMICAL ISOMERIZATION OF *o*-DI-*t*-BUTYLBENZENE IN ETHER

Time, hr.	Composition of isomeric di-*t*-butylbenzenes			Estimation method
	ortho	*meta*	*para*	
0	100%	. .	. . .	. . .
6	80	20%	Traces	Infrared
18	60	30	10%	Infrared
41	15	55	30	Infrared
64	5	50	45	Infrared
76	. . .	50	50	Infrared
112	. . .	30	70	N.m.r.
146	. . .	20	80	N.m.r.
158	. . .	20	80	N.m.r.

The same type of photoisomerization also occurs with *o*-*t*-butyltoluene, but at only one-fifth the above rate. However, after 36 hr. of irradiation, no isomerization could be detected with *o*- and *m*-xylene,[4a] *p*-*t*-butyltoluene, *o*-terphenyl, and 1,3,5-tri-*t*-butylbenzene. When a substituent such as nitro,[1] acetyl,[5] or methoxyl[5] was present in the 4-position of 1,2-di-*t*-butylbenzene, the isomerization was strongly inhibited.

In another experiment, irradiation of a mixture of *t*-butylbenzene and 1,3,5-*t*-butylbenzene failed to yield any detectable amount of di-*t*-butylbenzenes. Likewise, even after prolonged irradiation, none of the di-*t*-butylbenzenes showed any tendency to disproportionate into mono- and tri-*t*-butylbenzene.[6] Consequently, these photochemical isomerizations appear to be intramolecular in nature.

When the photoirradiation of *o*-di-*t*-butylbenzene was conducted in olefin-free petroleum ether (b.p. 35–40°), the rate of formation of *m*- and *p*-di-*t*-butylbenzene was much slower than in ether, and considerable amounts of colored by-products were also produced. Other experiments with photosensitizers[7] such as benzophenone revealed little dependency of the isomerization on the presence of such substances.

Mechanistically, these isomerizations can be accounted for either by photoinitiated 1,2-migrations of a *t*-butyl group or by excitation of the benzene ring to a Ladenburg-type structure with subsequent rearomatization to form the *meta* and thence the *para* isomer. We hope to distinguish these two possibilities by appropriate ring labeling with C^{14}, since there is no actual carbon–carbon migration of a *t*-butyl group in the latter pathway.

(1) A. W. Burgstahler, M. O. Abdel-Rahman, and P. L. Chien, *Tetrahedron Letters*, 61 (1964).
(2) E. E. van Tamelen and S. P. Pappas, *J. Am. Chem. Soc.*, **84**, 3789 (1962).
(3) With the use of a Vycor filter (No. 7913), only unidentifiable products, which darkened rapidly in air, could be obtained.
(4) After communication of these results to Professor van Tamelen, we were grateful to learn that he also has obtained similar findings in the photoirradiation of *o*-di-*t*-butylbenzene.
(4a) NOTE ADDED IN PROOF.—Since submission of this paper, related photoisomerizations of other dialkylbenzenes have been reported by K. E. Wilzbach and L. Kaplan [*J. Am. Chem. Soc.*, **86**, 2307 (1964)]. Using a much higher intensity light source, these workers observed isomerizations of *o*- and *m*-xylene which we have not been able to detect under our conditions, even after prolonged irradiation.
(5) Preparation described in the Ph.D. thesis of P. L. C., University of Kansas, 1964.
(6) Such disproportionation is well known in Friedel–Crafts reactions of di- and tri-*t*-butylbenzenes. *Cf.* P. D. Bartlett, M. Roha, and R. M. Stiles, *J. Am. Chem. Soc.*, **76**, 2349 (1954).
(7) *Cf.* J. Saltiel and G. S. Hammond, *ibid.*, **85**, 2515 (1963).