III was the predominant isomer. The product mixture arising from Ib that was 79–42% optically pure[10] showed no rotation at 578 nm (±0.003°). The CD spectrum of each mixture was identical (±0.2 millidegrees) with the base line from 400 to 250 nm (each mixture absorbed in this region). Compound IIIc separated from an ether solution of the reaction mixture.

As can be seen in Figure 1, the nmr spectrum of this compound in the presence of tris[3-(heptafluorobutyryl)-*d*-camphorato]europium(III) [Eu(hfbc)$_3$] showed a splitting of the enantiomeric methoxyl groups. IIc was produced from racemic, 46.3% optically pure (+)- and 38.6% optically pure (−)-Ib. The relative areas of the methoxyl signals arising from the two enantiomers were identical (±2%) in all cases.

The shifted spectra [Eu(hfbc)$_3$] of the mixture of benzoquinine adducts prepared from optically active Ib were identical (±5%) with similar spectra of the products from racemic Ib. If only half of the complex was decomposed in the presence of tetracyanoethylene, half of the initial rotation vanished. The recovered complex had not racemized. Consequently, at least greater than 95% of the cyclobutadiene ligand reacts after it has gained a plane of symmetry. This indicates that the metal was not close enough to the ligand to change its symmetry from that of the free ligand and should, therefore, have little effect on the free ligand's electronic state or reactivity.

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(10) For example, $[\alpha]_{578}$ 0.184°, 213 mg/6 ml of ether, 46.3% optically pure, and $[\alpha]_{578}$ −0.072°, 100 mg/6 ml of ether, 38.6% optically pure.

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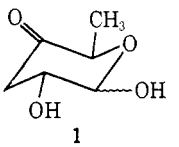
Synthesis of 3,6-Dideoxy-D-*erythro*-hexos-4-ulose and Identification as the 3,6-Dideoxy-4-ketohexose from *Pasteurella pseudotuberculosis*

Sir:

The 3,6-dideoxyhexoses are biologically important carbohydrates which contribute to the serological specificity of many immunologically active lipopolysaccharides.[1] Elucidation of the biochemical pathways for the formation of these sugars has been the subject of several recent investigations.[2,3] Abequose (3,6-dideoxy-D-*xylo*-hexose), paratose (3,6-dideoxy-D-*ribo*-hexose), and ascarylose (3,6-dideoxy-L-*arabino*-hexose) were shown to originate from CDP–6-deoxy-D-*xylo*-hexos-4-ulose (6-deoxy-4-keto-D-glucose) which in turn was formed from CDP–D-glucose.[4,5] The intermediate for the formation of paratose, abequose, and ascarylose from CDP–6-deoxy-4-keto-D-glucose was suggested to be 3,6-dideoxy-D-*erythro*-hexos-4-ulose (3,6-dideoxy-4-keto-D-glucose (**1**)) as its cytidine diphosphate nucleotide conjugate.[6] We now describe the synthesis of the free sugar **1** and its identification with the ketohexose isolated from *Pasteurella pseudotuberculosis* type V strain VO and thus unequivocally establish the structure of this important intermediate in the conversion of D-glucose into the 3,6-dideoxyhexoses. A recent report[7] of the occurrence of the L isomer of **1** (3,6-dideoxy-L-*erythro*-hexos-4-ulose) as part of the antibiotic cinerubine B provides added significance to the synthesis of **1**.



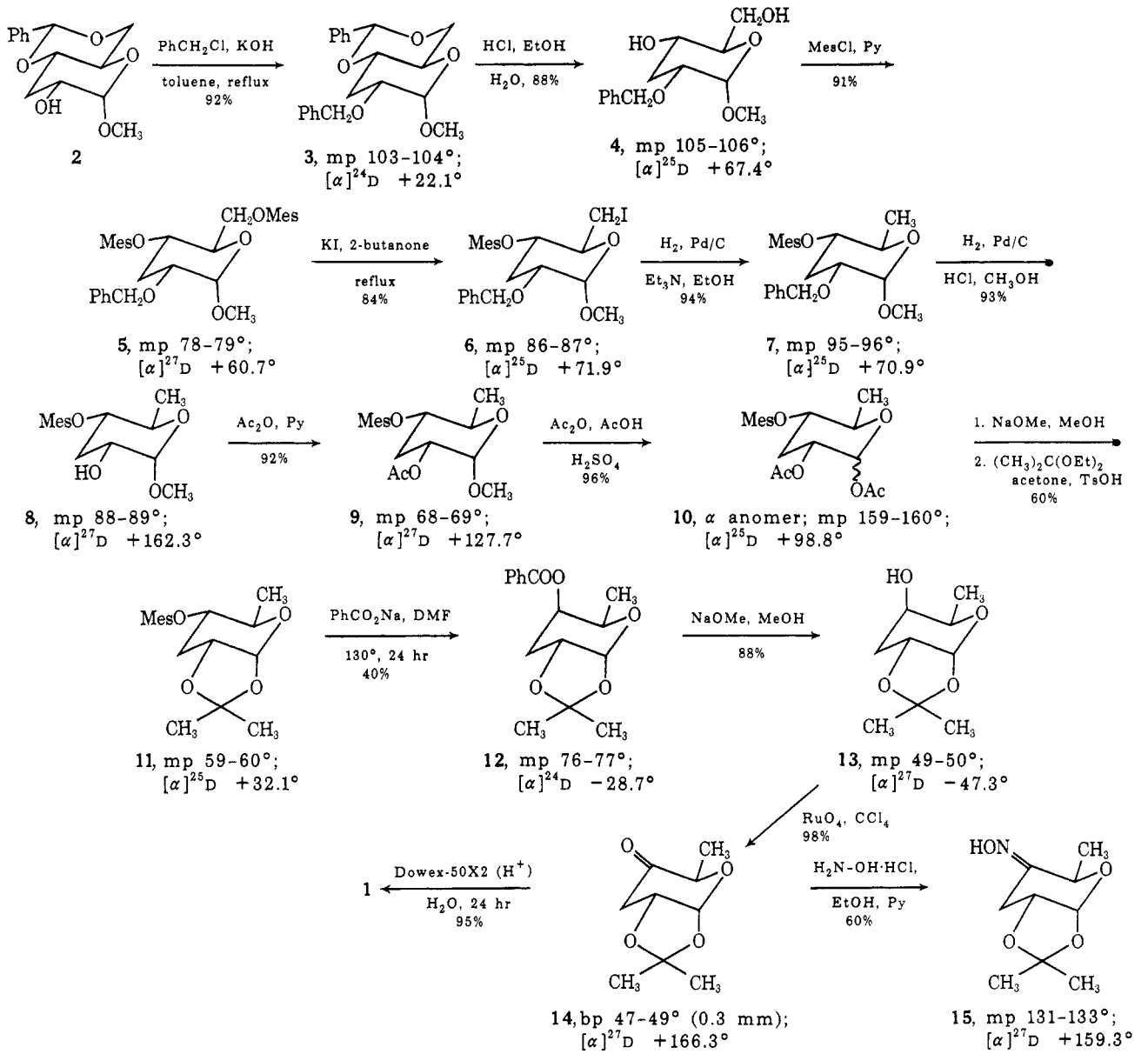
1

The synthesis of keto sugar **1** was accomplished starting from the known methyl 3-deoxy-4,6-*O*-benzylidene-α-D-*ribo*-hexopyranoside[8] (**2**) as summarized in Scheme I.[9] Free sugar **1**, which was obtained as an amorphous solid (nmr in DMSO-d_6–D_2O showed that **1** consisted of the α and β anomers in equal amounts) after lyophilization of the solvent, was converted to a crystalline derivative **15** for comparative studies with the natural product. This was achieved by converting **1** back to **14** by its treatment with 2,2-diethoxypropane in acetone in the presence of *p*-toluenesulfonic acid and the subsequent conversion of **14** to the crystalline oxime **15** in an overall yield of 25%.

The natural product uniformly labeled with ^{14}C was obtained by the following method. Enzymes E1, E3, and CDP glucose oxidoreductase were prepared from extracts of *Pasteurella pseudotuberculosis* type V strain VO as previously described.[2] CDP–6-deoxy-4-keto-D-glucose-*U*-^{14}C was prepared by treating CDP–glucose-*U*-^{14}C with CDP–glucose oxidoreductase in the presence of NAD^+.[10] After a small aliquot was treated with nucleotide pyrophosphatase and alkaline phosphatase, analysis by thin layer chromatography on silica gel F-254 using ethyl acetate–acetic acid–methanol–water (60:15:15:10) showed that the reaction had gone to about 95% completion. This material, without further purification, was treated with enzymes E1 and E3 in the presence of pyridoxamine 5′-phosphate and NADPH to obtain the CDP-3,6-dideoxy-4-ketohexose.[2,11] Analysis of the reaction by tlc on cellulose MN 300 using isobutyric acid–1 *M* ammonium hydroxide (5:3) showed that the reaction was more than 95% complete. The nucleotide was then purified by column chromatography on Bio-Rad AG1-X$_4$ (200–400 mesh) Cl^-, followed by gel filtration

(1) O. Luderitz, A. M. Staub, and O. Westphal, *Bacteriol. Rev.*, **30**, 192 (1966).
(2) P. Gonzalez-Porque and J. L. Strominger, *J. Biol. Chem.*, **247**, 6748 (1972), and references cited therein.
(3) For a review, see H. Nikaido and W. Z. Hassid, *Advan. Carbohyd. Chem.*, **26**, 351 (1971).
(4) S. Matsuhashi, M. Matsuhashi, and J. L. Strominger, *J. Biol. Chem.*, **241**, 4267 (1966).
(5) H. Nikaido and K. Nikaido, *ibid.*, **241**, 1376 (1966).
(6) S. Matsuhashi and J. L. Strominger, *ibid.*, **242**, 3494 (1967).
(7) W. Richle, E. K. Winkler, D. M. Hawley, M. Dobler, and W. Keller-Schierlein, *Helv. Chim. Acta*, **55**, 467 (1972).
(8) T. D. Inch and G. J. Lewis, *Carbohyd. Res.*, **15**, 1 (1970); E. J. Hedgley, W. G. Overend, and R. A. C. Ronnie, *J. Chem. Soc.*, 4701 (1963).
(9) The melting points are uncorrected. The specific rotations were obtained in chloroform solutions with a concentration of *ca.* 1 g/100 ml. All new compounds, except **1**, gave satisfactory elemental analysis.
(10) CDP-D-glucose-*U*-^{14}C, 200 mCi/mmol, was purchased from ICN. Pyridoxamine 5′-phosphate hydrochloride, NAD^+, NADPH, nucleotide pyrophosphatase, and *Escherichia coli* alkaline phosphatase were purchased from Sigma Chemical Co.
(11) P. Gonzalez-Porque and J. L. Strominger, *Proc. Nat. Acad. Sci. U. S.*, **69**, 1625 (1972).

Scheme I



on a column of Bio-Gel P2. The free ketosugar-*U*-^{14}C **1** was obtained by subjecting the above purified solution to enzymatic treatment with alkaline phosphatase and nucleotide pyrophosphatase. The resulting solution, without further purification, was used for comparison with the synthetic material.

An aqueous solution of the free sugar **1** was prepared by hydrolysis of 95 mg of **14** as described earlier. A sample of the natural product containing 295,000 dpm[12] was combined with the above solution and the mixture was freeze-dried at 0.02 mm. The resulting solid was converted to the oxime **15** as described before and was purified by preparative tlc on silica gel G using an ethyl acetate–benzene (1:1) system. Recrystallization from hexane provided 20 mg of the crystalline material having 2240 dpm/mg which accounted for 88% of the activity of the original sample. Subsequent recrystallizations (twice each from hexane and methanol–water) did not change the disintegrations per minute of the oxime, showing that the synthetic material and natural product were identical. Supporting evidence was obtained by radioautography[13] on silica gel G thin layer plates in three different solvent systems, acetone, ethyl acetate, and 2-propanol. The R_f of the synthetic material in each case corresponded to the major spot of the natural product as shown by its radioactivity.

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(12) A Packard Model 2002 Tri-Carb liquid scintillation spectrometer was used for measuring the disintegrations per minute. The authors are grateful to Dr. H. C. McAllister for his help and cooperation in the use of this instrument.

(13) The authors thank Dr. T. T. Tchen and his research group for their help in the use of the Nuclear-Chicago Model Isocap/300 liquid scintillation system for the radioautography.

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