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2. In C_3 plants, carbon dioxide is fixed into a three-carbon sugar. This fixation and the subsequent metabolic cycle is known as the Calvin cycle. C_4 plants fix carbon dioxide into a four-carbon compound such as malic or aspartic acid, which is subsequently decarboxylated. The carbon dioxide then enters the Calvin cycle as in a C_3 plant. Stomatal opening and carbon dioxide uptake occur during the day in C_3 and C_4 plants. In contrast, carbon dioxide is fixed into malic acid during the night in CAM plants. The malic acid is then decarboxylated during the day and the carbon dioxide enters the Calvin cycle as in a C_3 plant.
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8. The δ values are defined as

$$[(R_{sample}/R_{standard}) - 1] \times 1000$$

where $R = {}^{18}O/{}^{16}O$ for $\delta^{18}O$, R = deuterium/hydrogen for δD, and $R = {}^{13}C/{}^{12}C$ for $\delta^{13}C$. The standard is standard mean ocean water (SMOW) for hydrogen and oxygen and the Peedee belemnite (PDB) for carbon.
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20. We thank H. Ajie for technical assistance and D. Winter for performing the isotopic measurements. Supported by NSF grant ATM 79-24581.

* M.J.D. is also in the Archaeology Program.

5 October 1982; revised 7 December 1982

Total Synthesis of the L-Hexoses

Abstract. *Enantiomerically pure polyhydroxylated natural products are synthesized by using a reiterative two-carbon extension cycle consisting of four steps. The generality and efficiency of this methodology are demonstrated in the total synthesis of all eight L-hexoses.*

We describe here the systematic, stereoselective synthesis of all eight L-hexoses by a synthetic methodology developed in our laboratories for the preparation of polyhydroxylated natural products (*1*). Most monosaccharide syntheses have involved modification of sugars that occur naturally (*2*), and recorded total syntheses have usually been carried out in a racemic form and with poor stereoselection (*3*). The stereochemical challenge involved in a general synthesis of monosaccharides, though purely academic, has now been met, and a high degree of stereocontrol is attainable (*4–6*).

Our strategy is based on the reiterative two-carbon extension cycle, which consists of four steps (Fig. 1): I, conversion of an aldehyde into its corresponding *E*-allylic alcohol; II, asymmetric epoxidation (AE) with titanium tetraisopropoxide, *t*-butylhydroperoxide, and diethyl (+)- or (−)-tartrate; III, treatment of the epoxy alcohol with benzenethiolate anion in a basic medium; and IV, oxidation and Pummerer reaction of the sulfide followed by the net hydrolysis of the resulting *gem*-acetoxysulfide with or without inversion of the C(2) center (*7*). Because of the presence of four hydroxymethylene centers in the hexoses, the synthesis of these compounds requires a double application of the basic cycle.

The synthesis described here begins with a single fundamental building block, 4-benzhydryloxy-(*E*)-but-2-en-1-ol, **1**, a compound which is readily prepared from (*Z*)-2-butene-1,4-diol (*8*). Step I of the extension cycle is therefore eliminated in this initial case. The selection of the benzhydryl protecting group rather than a more common group such as benzyl has proved to be critical (see below), and the benzhydryl serves its purpose through the entire synthesis, which is shown in Fig. 2 with the yield and selectivity for each step. Conversion of **1** into **4** and **5** completes the first cycle, and the conversion of **4** and **5** into **16** to **23** constitutes the entire set of the second cycle. A succinct explanation of Fig. 2 with comments is given below with the routes leading to L-allose (**16**) and L-altrose (**17**) as examples.

(Conversion of **1** → **4**) Sulfide **3** is prepared in the manner described for the case of R = benzyl (*5*). Oxidation of **3** to the sulfoxide followed by a Pummerer rearrangement under Tsuchihashi conditions (*9, 10*) provides the *gem*-acetoxysulfide intermediate, which is reduced with Dibal at −78°C to give the aldehyde **4** in overall 84 percent yield. The epimeric aldehyde **5** is absent. (**4** → **6b**) Homologation of **4** with formylmethylenetriphenylphosphorane followed by reduction with sodium borohydride leads to allylic alcohol **6b** in 88 percent yield and with excellent selectivity. This compound is ready for application of the second AE. (**6b** → **8**) In earlier experiments in which the substrate **6b′** was used with R = benzyl, the AE reaction was accompanied by a subsequent titanium-catalyzed epoxide opening. This process involved participation by the oxygen atom of the C(6) benzyloxy group, which led to formation of the undesired corresponding tetrahydrofuran. Use of **6b**, however, prevents this ethereal oxygen participation and AE proceeds smoothly. The selectivity of this reaction appears perfect, as no trace of the diastereoisomer **9** is detected by the usual techniques of analysis. (**8** → **16**) Treatment of epoxy alcohol **8** with benzenethiolate anion in an alkaline medium results in the isolation of a diol (77 percent), which is quantitatively transformed into **12**. A sequence of high-yielding steps—(i) oxidation and Pummerer rearrangement (90 percent) and (ii) Dibal reduction (81 percent), deprotection with trifluoroacetic acid, and catalytic hydrogenation (90 percent)—affords the free sugar L-allose (**16**). Alternatively, potassium carbonate in metha-

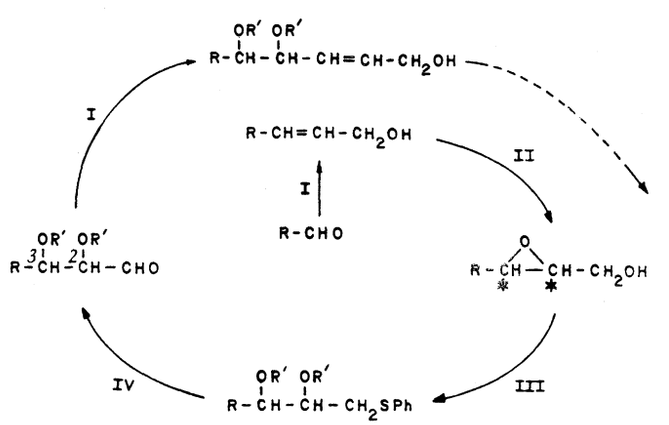

Fig. 1. Reiterative two-carbon extension cycle.

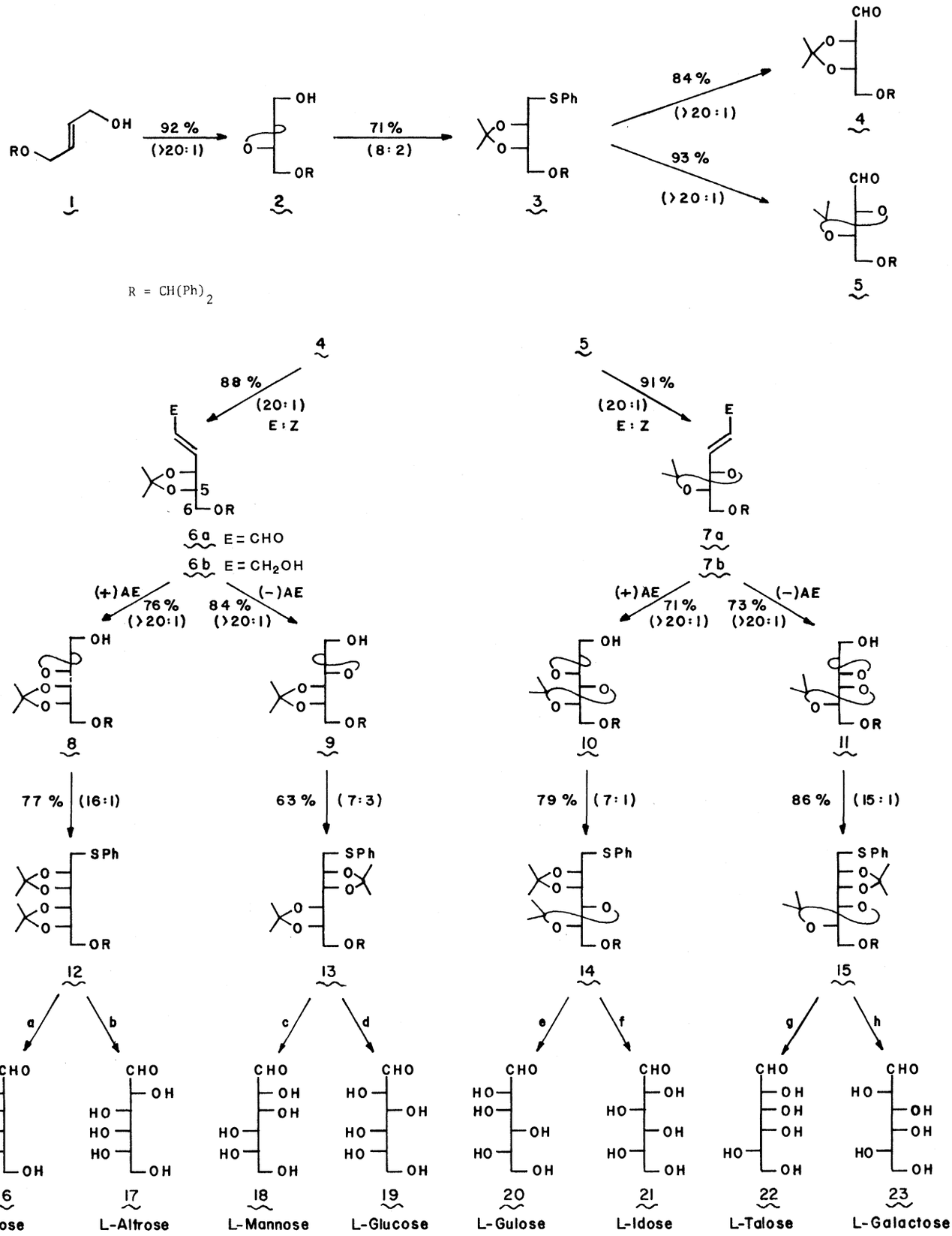

Fig. 2. Synthesis of L-hexoses. For a, c, e, and g, 1 = Pummerer reaction, 2 = Dibal, 3 = deprotection. a: 1 (90 percent), 2 (81 percent), 3 (90 percent). c: 1 (90 percent), 2 (95 percent), 3 (90 percent). e: 1 (87 percent), 2 (81 percent), 3 (84 percent). g: 1 (71 percent), 2 (77 percent), 3 (61 percent). For b, d, f, and h: 1 = Pummerer reaction, 2 = potassium carbonate and methanol, 3 = deprotection. b: 1 (90 percent), 2 (48 percent), 3 [see (*11*)]. d: 1 (90 percent), 2 (60 percent), 3 (20 percent). f: 1 (87 percent), 2 (66 percent), 3 (85 percent). h: 1 (71 percent), 2 (41 percent), 3 (27 percent).

nol is substituted for Dibal in step (ii), followed by deprotection. This results in the complete epimerization of the C(2) center (*7*), and provides L-altrose (**17**) (*11*).

The sequences leading to the other hexoses have also been carried out satisfactorily in the manner described above for L-allose and L-altrose (*12*). All steps in Fig. 2 except for the step **9** → **13** proceed with remarkable regio- and stereoselection. Since the mirror image of every compound in Fig. 2 can be prepared by simple exchange of the chiral ligand (tartrate ester) in the AE reaction, the formal synthesis of the D-hexoses has also been achieved. Thus, our two-carbon extension methodology has proved to be generally applicable and efficient in controlling stereochemistry in the construction of acyclic, polyhydroxylated carbon frameworks.

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References and Notes

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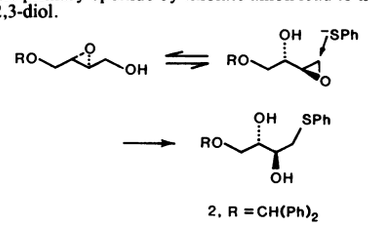
7. See Lee *et al.* (*6*). Although the hydrolysis with epimerization of the 4-carbon Pummerer product proceeds rapidly, completely, and in excellent yield, some complications are observed in the 6-carbon case. In general, this hydrolysis with epimerization proceeds in somewhat lower yield, primarily due to instability and difficulties with isolating the partially hydrated aldehyde product. The epimerization, however, proceeds to give an isomer ratio of > 95:5 in all these 6-carbon cases.
8. Available from Aldrich Chemical Co.
9. See Lee *et al.* (*6*) and references cited therein. Treatment of epoxy alcohol **2** with 0.5*N* NaOH (2.5 equivalents) in *t*-butyl alcohol followed by slow addition of thiophenol (1.2 equivalents) in moist *t*-butyl alcohol and subsequent trapping of the primary epoxide by thiolate anion lead to the 2,3-diol.



2, R = $CH(Ph)_2$

Treatment of the newly formed diol with 2-methoxypropene in the presence of a catalytic amount of camphorsulfonic acid provides **3** in 71 percent overall yield with a 4:1 selectivity.
10. S. Iriuchijima, K. Maniwa, G. Tsuchihashi, *J. Am. Chem. Soc.* **96**, 4280 (1974).
11. Attempts to deprotect the L-altrose derivative with trifluoroacetic acid, followed by catalytic hydrogenation, invariably gave 1,6-anhydro-β-L-altropyranose, which is known to exist in acid solutions in equilibrium with the free sugar [S. Peat, in *Advances in Carbohydrate Chemistry*, W. W. Pigman and M. L. Wolfram, Eds. (Academic Press, New York, 1946), vol. 2, p. 38]. Exposure of commercially obtained D-altrose to acid also produced the 1,6-anhydro-β-D-altropyranose, which upon acetylation proved identical to the synthetic peracetylated β-L-1,6-anhydro derivative.
12. All eight synthetic L-hexoses had identical mobility on thin-layer chromatographic plates and had identical 270-MHz nuclear magnetic resonance (NMR) spectra in D_2O (except for slight differences) as commercially obtained samples of the D- or L-hexoses. In addition, all eight aldehydes (**16** to **23** in Fig. 2) were reduced and successfully transformed into their corresponding peracetylated hexitols, six out of the seven (altrose and talose give the same hexitol) of which were previously known. The synthetic hexitol acetates were judged on the basis of infrared spectra, NMR, melting points, and optical rotation (where applicable) to be identical with the naturally derived materials. This transformation unequivocally confirms the stereochemistry of the critical C(2) through C(5) centers. We were, however, unable to obtain satisfactory optical rotations for several of the synthetic L-hexoses, due primarily to the inaccuracies associated with weighing small quantities of hydrated samples. These results will be reported in full elsewhere.

13. We are grateful to the National Institutes of Health (grant GM 31124) and to the National Science Foundation for financial support. High-resolution mass spectra were provided by a facility supported by the National Institutes of Health (grant RR 00317).

8 November 1982

Hemoglobin in a Nonleguminous Plant, *Parasponia*: Possible Genetic Origin and Function in Nitrogen Fixation

Abstract. *A dimeric hemoglobin was purified from nitrogen-fixing root nodules formed by association of* Rhizobium *with a nonleguminous plant,* Parasponia. *The oxygen dissociation rate constant is probably sufficiently high to allow* Parasponia *hemoglobin to function in a fashion similar to that of leghemoglobin, by oxygen buffering and transport during symbiotic nitrogen fixation. The identification of hemoglobin in a nonlegume raises important questions about the evolution of plant hemoglobin genes.*

In higher plants, hemoglobin is generally thought to occur only in the nitrogen-fixing root nodules of legumes (*1*). Because the structure of legume hemoglobin (leghemoglobin) genes is very similar to that of animal globin genes, it has been suggested that the gene for leghemoglobin was transferred to legumes from another eukaryote outside the plant kingdom, relatively recently in evolutionary history (*2*). We report that hemoglobin also occurs in the nitrogen-fixing root nodules of *Parasponia*, a member of the Ulmaceae. This plant is nodulated by strains of *Rhizobium* that also nodulate certain members of the Leguminosae (*3*).

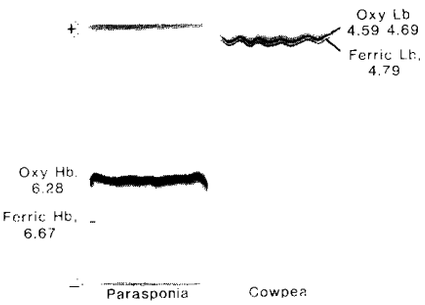


Fig. 1. Preparative-scale isoelectric focusing of oxyhemoglobin (*Oxy Hb*) from *P. andersonii* nodules and leghemoglobin (*Lb*) from cowpea [*Vigna unguiculata* (L.) Walp.] nodules, both inoculated with *Rhizobium* strain CP283 and grown as previously described (*3*). Focusing was performed at 4°C in a 100-ml gel slurry bed of Ultrodex (LKB) containing 0.5 percent Ampholine (LKB) (*p*H 4.0 to 6.0) and 1.5 percent Ampholine (*p*H 5.0 to 7.0) (*14*). The gel plate was loaded with approximately equal amounts of the total hemoglobin purified by Sephacryl S200 chromatography from *Parasponia* and cowpea nodule extracts. Isoelectric points, measured at 4°C, are marked.

The absorption spectra of *Parasponia* nodule segments are consistent with the presence of hemoglobin (*4*), and this *Parasponia* hemoglobin may be purified from nodules extracted under strict anaerobic conditions that prevent tannin formation and hemoglobin degradation. Fresh nodules from *Parasponia andersonii* Planch, infected with *Rhizobium* strain CP283 (*3*), or nodules frozen in liquid N_2 were dropped into the steel chamber of an Omnimixer (Sorvall) containing four volumes of extraction buffer [50 m*M* potassium phosphate (*p*H 7.2), 1m*M* EDTA, 4 percent soluble polyvinylpyrrolidone (Kollidon 25, BASF), and 0.1 percent sodium dithionite (Fluka)] equilibrated with pure CO. The mixture was ground at full speed under CO for 2 minutes at 0°C, then centrifuged under CO at 100,000*g* for 60 minutes at 0°C. The yield of crude carboxyhemoglobin was 45 to 70 nanomoles per gram of *Parasponia* nodule tissue (*5*). Anaerobic chromatography on a column of Sephacryl S200 (Pharmacia) equilibrated with CO-saturated 50 m*M* potassium phosphate and 0.1 m*M* EDTA (*p*H 7.2) at 0°C