

# Synthesizing Labeled Compounds

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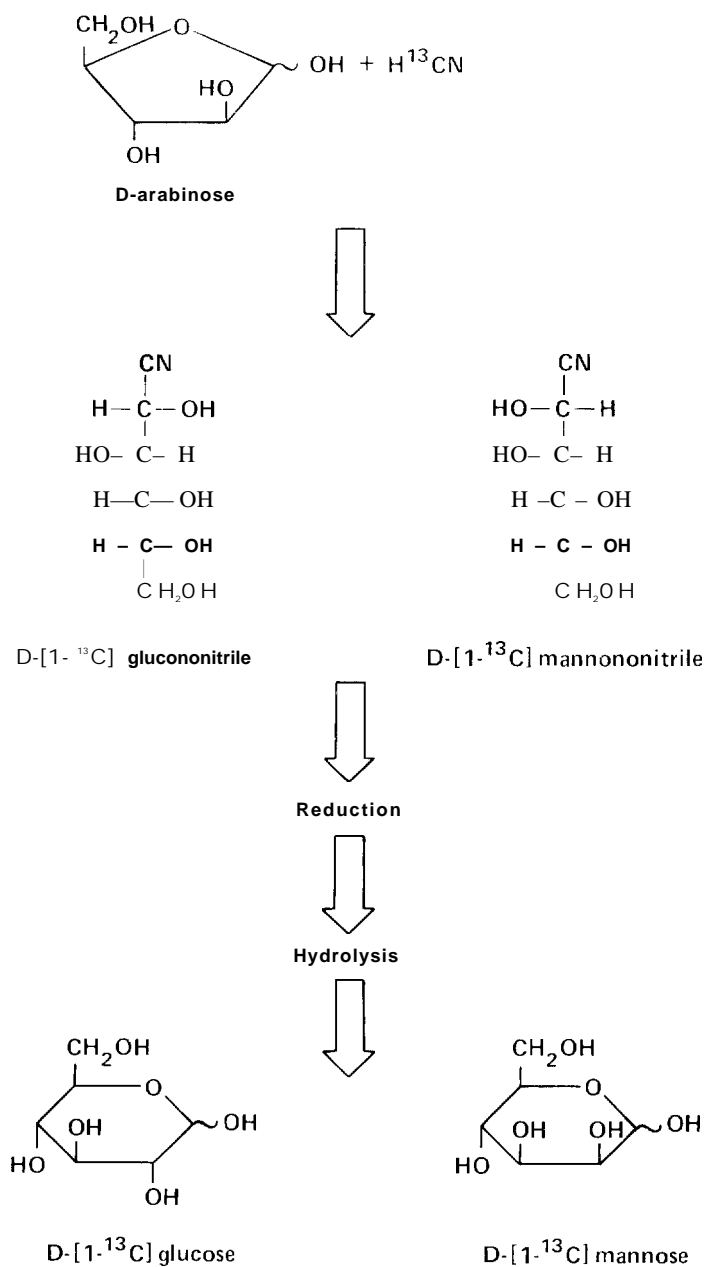
## RELATED WORK

**A**mong the applications for the Laboratory's harvest of carbon monoxide enriched in carbon-13 are metabolic studies based on NMR spectroscopy. But first the isotope and its nuclear magnetic moment label must be incorporated at chosen sites in biochemical substances such as sugars and amino acids. The Laboratory has pioneered in developing chemical and biochemical methods for accomplishing this often intricate task.

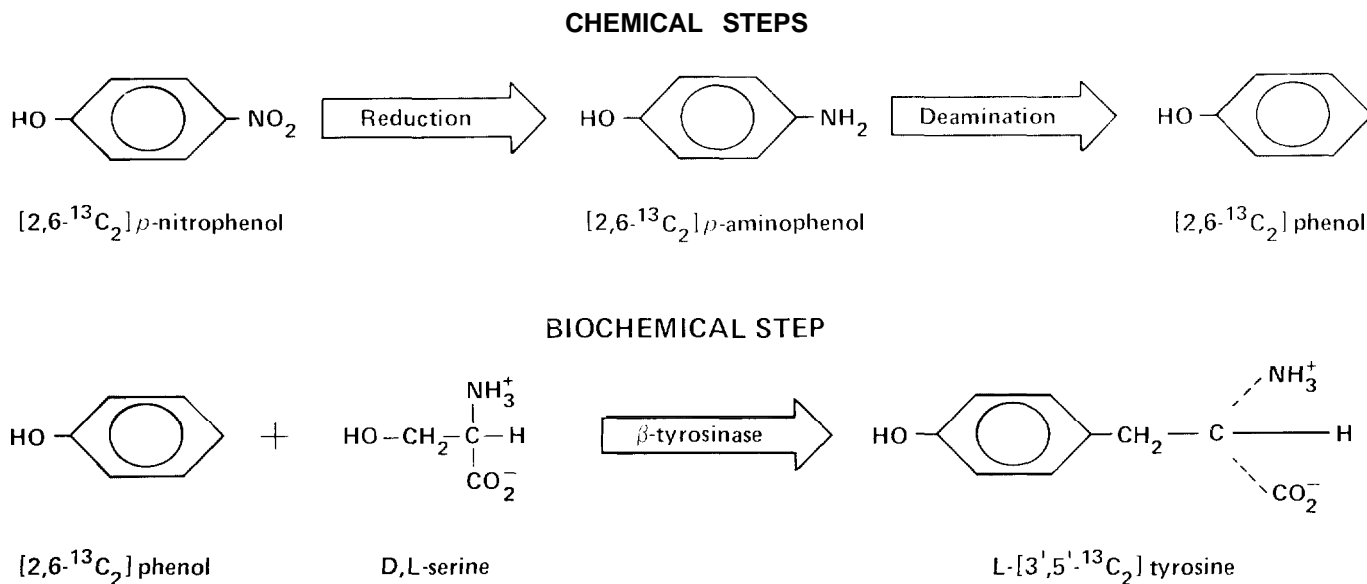
For example, T. W. Whaley and R. M. Blazer of Los Alamos and Robert Barker and coworkers of Cornell University devised a chemical process (Fig. 1) for preparing a labeled form of the most common sugar, D-glucose. The essential reactant is  $^{13}\text{C}$ -labeled hydrogen cyanide ( $\text{H}^{13}\text{CN}$ ), which is obtained from  $^{13}\text{C}$ -enriched carbon monoxide by the reactions  $^{13}\text{CO} + \text{H}_2 \xrightarrow{\text{Ni}} ^{13}\text{CH}_4$  and  $^{13}\text{CH}_4 + \text{NH}_3 \xrightarrow{\text{Pt}} \text{H}^{13}\text{CN}$ . The labeled hydrogen cyanide is then reacted with the 5-carbon sugar D-arabinose to form a mixture of D-[1- $^{13}\text{C}$ ]glucononitrile and D-[1- $^{13}\text{C}$ ]mannononitrile, which is then converted to a mixture of D-[1- $^{13}\text{C}$ ]glucose and D-[1- $^{13}\text{C}$ ]mannose.

Since D-mannose is of limited utility and yet forms the bulk (75 percent) of the product, considerable effort has been directed to investigating various means for catalyzing its conversion to D-glucose, Barker and coworkers found recently that molybdate ion ( $\text{MoO}_4^{2-}$ ) is an effective catalyst, but NMR studies showed unequivocally that this catalyst converts D-[1- $^{13}\text{C}$ ]mannose to D-[1- $^{13}\text{C}$ ]glucose rather than the expected D-[1- $^{13}\text{C}$ ]glucose. This result is but one example of the unique insights into the course of chemical reactions provided by isotopic labeling and NMR spectroscopy.

Chemical syntheses such as the Barker process are sometimes the only practical route to a particular labeled compound but are often beset by difficulties: many are multi-step and hence lengthy, labor-intensive, and costly, and the yield of the labeled



**Fig. 1.** Synthesis of  $^{13}\text{C}$ -labeled D-glucose, a 6-carbon sugar, involves adding a labeled nitrile group to the 5-carbon sugar D-arabinose by reaction with labeled hydrogen cyanide. The product of this reaction, a mixture of labeled nitriles of D-glucose and D-mannose (another 6-carbon sugar), is then reduced and hydrolyzed to a mixture of the labeled sugars. The two sugars are separated by absorption chromatography.



**Fig. 2.** Synthesis of  $^{13}\text{C}$ -labeled L-tyrosine, an amino acid, is accomplished in only three steps. The third and simplifying step, the reaction between labeled phenol and the amino acid serine, capitalizes on the ability of the bacterium *Erwinia*

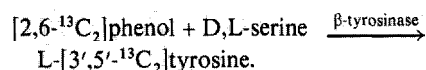
*herbicola* to produce large quantities of the necessary enzyme catalyst. Note that the reaction yields L-serine irrespective of whether the enzyme acts on D-serine, L-serine, or a mixture of the two isomers.

product may be disappointingly low because of diversion of the isotope into undesired products or positions. Since biochemical syntheses are often less subject to these difficulties, they are being pursued at Los Alamos—and with noteworthy success.

To illustrate, consider the synthesis of a labeled form of the amino acid L-tyrosine, which is present in nearly all proteins and is a precursor of the hormones epinephrine (adrenalin), norepinephrine, and thyroxine. V. J. Hruby of the University of Arizona has developed a ten-step chemical process for synthesizing a mixture of the D and L isomers of  $[3',5'-^{13}\text{C}_2]$ tyrosine from labeled *p*-nitrophenol. Based on the carbon-13 contained in the *p*-nitrophenol, the yield of the isomeric mixture is about 30 percent; separating the biologically active L isomer reduces the yield to 14 percent.

In contrast, T. E. Walker of Los Alamos and C. B. Storm of Howard University developed a combined chemical-biochemical method for preparing L- $[3',5'-^{13}\text{C}_2]$ tyrosine from the same labeled reactant in three steps with a yield of 80 percent. As in the synthesis of D-glucose, the labeled reactant, in this case *p*-nitrophenol, has its origins in  $^{13}\text{C}$ -enriched carbon monoxide:  $^{13}\text{CO} + \text{O}_2 \rightarrow ^{13}\text{CO}_2 \xrightarrow{\text{Cu,Cr,Zn}} ^{13}\text{CH}_3\text{OH}, ^{13}\text{CH}_2\text{OH} + \text{CO} \xrightarrow{\text{HI,Rh}} ^{13}\text{CH}_3\text{COOH}, ^{13}\text{CH}_2\text{COOH} + \text{Ba(OH)}_2 \rightarrow \text{Ba}(^{13}\text{CH}_2\text{COO})_2 \xrightarrow{\text{pyrolysis}} ^{13}\text{CH}_3\text{CO}^{13}\text{CH}_3 \xrightarrow{\text{condensation}} [2,6-^{13}\text{C}_2]p\text{-nitrophenol}$ . The tyrosine synthesis (Fig. 2) in-

volves two chemical steps that convert *p*-nitrophenol to phenol followed by a key biochemical step that converts phenol directly to L-tyrosine with the aid of the bacterium *Erwinia herbicola*. Under suitable conditions this microorganism manufactures high levels (up to 10 percent of its cell protein) of the enzyme  $\beta$ -tyrosinase. (This property was discovered in the course of Japanese research and development directed toward the use of microorganisms to produce large quantities of amino acids as food supplements.) After filling themselves with a surfeit of the enzyme, the bacteria are placed in a medium containing the labeled phenol and D-wine, L-serine, or a mixture of both, and the enzyme catalyzes the reaction



*E. herbicola's* generous supply of  $\beta$ -tyrosinase yields other labeled forms of L-tyrosine from variations on this reaction: L-[1-, 2-, or 3- $^{13}\text{C}$ ]tyrosine from unlabeled phenol and D, L-[1-, 2-, or 3- $^{13}\text{C}$ ]serine; and  $^{15}\text{N}$ -labeled L-tyrosine from unlabeled phenol unlabeled pyruvate, and  $^{14}\text{N}$ -labeled ammonia. (Nitrogen-15 is another isotope useful in metabolic studies based on NMR spectroscopy.)

Another example of biochemical synthesis is the use of the metabolically defective microorganism *Brevibacterium flavum* to

produce L-[2,4- $^{13}\text{C}_2$ ] glutamate in high yield from D-[1- $^{13}\text{C}$ ] glucose. The route to this amino acid (a salt of which, monosodium glutamate, is well known as the seasoning MSG) involves  $\alpha$ -ketoglutarate, one of the intermediates in the Krebs cycle. In normal organisms  $\alpha$ -ketoglutarate progresses through the Krebs cycle to succinate by action of the enzyme  $\alpha$ -ketoglutarate dehydrogenase. *B. flavum*, however, produces very little of the enzyme, and when these bacteria are grown in a medium containing glucose,  $\alpha$ -ketoglutarate accumulates and is converted, in the presence of ammonium ion, to L-glutamate. A sick but kindly organism, *B. flavum* suffers from the additional disorder of a leaky cell membrane, which allows passage of the glutamate into the medium where it can be recovered readily.

On the horizon for the biochemical synthesis program at Los Alamos is the use of recombinant DNA techniques to genetically engineer microorganisms with optimal properties for the production of labeled biochemical substances. By concurrently probing these microorganisms *in vivo* with NMR techniques, the metabolic consequences of the genetic engineering can be ascertained. This approach, being taken by C. J. Unkefer of Los Alamos in collaboration with J. K. Griffith of the University of New Mexico, may be one of the most significant research directions in stable isotope technology in this decade. ■