PROCEEDINGS OF THE FIRST INTERNATIONAL CONFERENCE ON STABLE ISOTOPES IN CHEMISTRY, BIOLOGY, AND MEDICINE

May 9-11, 1973

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and

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CLINICAL APPLICATIONS OF STABLE ISOTOPES

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INTRODUCTION

There has been a striking increase in the use of stable isotopes in the last five years. In part, this is due to anticipated increases in the availability of ^{13}C, ^{15}N, ^{18}O and the depleted forms of ^{12}C and ^{14}N through the production facilities at the USAEC's Los Alamos Scientific Laboratory. In part, it is also due to the preparation and investigation of ^{2}H- and ^{13}C-labeled compounds by nuclear magnetic resonance, electron spin resonance and electron nuclear double resonance spectroscopy. Still another sector of interest arises from gas chromatographic-mass spectrometric techniques employing stable isotopes in studies of drug metabolism. The absence of radiation hazard and the consequent applicability of stable isotopes to clinical problems of infants, children and pregnant women is perhaps one of the strongest factors in the growth of this field. In addition to these areas, interest is strong in fundamental studies of isotope effects *in vitro* and *in vivo*, new developments in stable isotope instrumentations and stable isotope applications in agricultural and environmental research.

This diversity of interest has led in the past to small meetings on individual topics, but there has been no attempt prior to this meeting to bring together the entire community of stable isotope users in one encompassing conference. This was the objective of the Organizing Committee and, as is evident from the size of these Proceedings, the response was widespread and enthusiastic. More than 240 registrants contributed a total of 64 papers to the three day meeting and of these, more than 50 are represented by manuscripts in the Proceedings. Equally important, the community of stable isotope users became visible as an entity. This community has overlapping and adjacent objectives, shares a common interest in this broad spectrum topic and interacts across disciplinary boundaries. It is hoped that having once been convened, this group will again meet after an appropriate interval, and that those who find in these Proceedings a link to their own research will join them in the next Conference on Stable Isotopes in Chemistry, Biology and Medicine.

Warm thanks are due to Merck, Sharp and Dohme, Canada, Ltd. and to G. D. Searle & Company for their generous support of the Conference operation. Additional support was provided by Argonne National Laboratory through its Chemistry Division, the Division of Biological and Medical Research and the Center for Educational Affairs, by the U.S. Atomic Energy Commission, and by the University of Chicago. The Organizing Committee acknowledges all of these sponsors with deep appreciation and particularly thanks Ms. Beverly Litt, Chemistry Division, and Mrs. Dorothy Carlson, Conference Planning Office, for their many contributions to the arrangement of the Conference.

Henry L. Crespi
Joseph J. Katz
Peter D. Klein

SESSION I

SYNTHESES WITH STABLE ISOTOPES

Chairman: J. Fried
University of Chicago

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ORGANIC SYNTHESIS WITH STABLE ISOTOPES

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INTRODUCTION

The use of stable isotopes in chemistry, biology, and medicine is almost totally dependent on their availability in a variety of chemical forms (1). The compounds available directly or indirectly from various isotope enrichment processes are generally simple molecules such as carbon monoxide, carbon dioxide, nitric oxide, water, and ammonia. Since this small number of isotopic molecules would severely limit the types of experiments that can be performed with stable isotopes, it is necessary for the synthetic chemist to devise methods for the conversion of simple molecules into more complex compounds which are of greater interest to people in the physical and life sciences.

These chemical transformations can be accomplished by utilizing biosynthetic systems, organic synthetic methods, or by a combination of both methods (2). Naturally occurring substances which are uniformly labeled with stable isotopes are most easily prepared by biosynthesis. Compounds which contain specific site labels are generally obtained by chemical synthesis. Combinations of organic synthesis and biosynthesis offer distinct advantages for preparation of labeled molecules. For example, specifically labeled compounds can be used as biosynthetic precursors, and, in the opposite sense, a uniformly labeled compound prepared by biosynthesis can be the most readily available starting material for an organic synthesis. However, the scope of this paper will be limited to a discussion of some of our work in preparing labeled compounds by organic synthesis.

DISCUSSION

While there are many similarities between syntheses with stable isotopes and radioisotopes, there are significant differences also. Perhaps the major difference is that of reaction scale. Reactions with stable isotopes are generally run on a larger scale than their radioactive counterparts. Thus, many of the elegant semimicro techniques developed for radiosynthesis cannot be applied to stable isotope preparations. Syntheses with stable isotopes tend to rely more on the conventional methodology of organic chemistry and, in the present case, even resemble small-scale industrial preparations. In addition to affecting manipulative synthesis techniques, larger reaction scales also restrict purification techniques. Hence, conventional methods of distillation, crystallization, and sublimation are used more frequently than techniques such as chromatographic purification.

The degree of enrichment utilized in stable isotope preparations also produces effects which are not usually encountered in analogous radioisotope work. Most preparations involving radioisotopes contain sufficient carrier

material such that physical and spectral properties of the radioactive mixture are essentially those of the nonradioactive compound. However, in the case of compounds labeled with stable isotopes, even moderate levels of enrichment can have discernible effects on physical properties (e.g., molecular weight, density) and spectral properties (e.g., ir, nmr) of the labeled compound. Figure 1 shows the effect of carbon-13 incorporation (ca. 90% enrichment) on the methyl resonance of the ^{1}H-nmr spectrum of acetic acid. The figure also demonstrates the necessity for considering isotope isomers when stable isotope enrichments are less than 100%. For example, when acetic-1,2-$^{13}C_2$ acid is prepared from 90% carbon-13, only 81% of the molecules are truly doubly labeled, while 9% are labeled in the methyl group, 9% are labeled in the carboxyl group, and 2% contain only carbon-12.

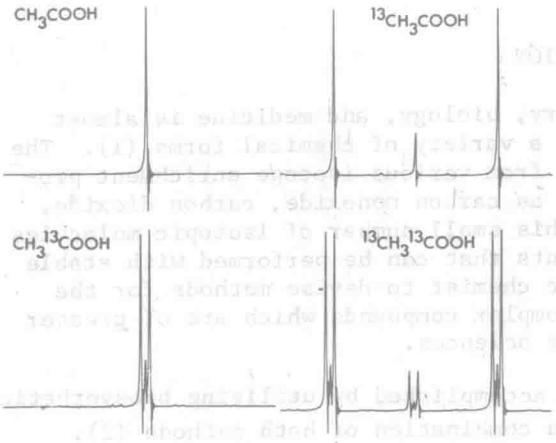

Fig. 1.--Proton nuclear magnetic resonance spectra of isotope isomers of acetic acid containing 90 atom % carbon-13

The isotope production facility at the Los Alamos Scientific Laboratory currently produces the following nuclides (3): carbon-13 (ca. 90% ^{13}C), carbon-12 (99.999% ^{12}C), nitrogen-15 (99% ^{15}N), nitrogen-14 (99.995% ^{14}N), oxygen-16 (99.999% ^{16}O), oxygen-17 (ca. 90% ^{17}O), and oxygen-18 (99% ^{18}O). The carbon isotopes, produced by the low temperature distillation of carbon monoxide, are available as either carbon monoxide or carbon dioxide. The nitrogen and oxygen isotopes, produced by a similar distillation of nitric oxide, are generally available as ammonia and water, respectively.

Our initial synthetic work involved the large-scale production of acetic acid to be used as a carbon source in a mouse-feeding experiment designed to demonstrate the lack of toxic effects with high levels of carbon-13 (4). The acetic acid was prepared by the following sequence of reactions:

$$CO_2 \xrightarrow[\text{Cu-Zn-Cr}]{H_2} CH_3OH \xrightarrow[\text{HI, 175°}]{CO,\ RhCl_3} CH_3COOH$$

In addition to providing acetic acid for the mouse-feeding experiment, this work established our ability to synthesize large quantities (ca. 5 moles/day) of two intermediate compounds (methanol and acetic acid) which could be used in other syntheses.

A large portion of our current work has been devoted to preparing compounds containing enriched levels of stable isotopes to be used in various collaborative experiments. While our ultimate goal has been to prepare labeled molecules

for chemical, biological, and clinical purposes, we have also concentrated on developing a nucleus of simple intermediates that can be used to prepare a large variety of labeled compounds. Our initial synthetic sequence of carbon dioxide-methanol-acetic acid has served as a backbone for constructing a larger synthetic framework of simple intermediates and labeled compounds.

METHODS AND RESULTS

Syntheses from Carbon Monoxide (Fig. 2):--Carbon dioxide is routinely prepared by the cupric oxide oxidation of carbon monoxide. A second useful intermediate, isopropyl formate, has been obtained by allowing carbon monoxide to react with isopropyl alcohol containing a catalytic amount of sodium isopropoxide at room temperature. This equilibrium reaction requires moderate pressure but has been carried out using standard laboratory equipment (5). A mixture of carbon monoxide, ammonia, and sulfur in methanol has been converted to urea in a low-pressure apparatus at 100° (6). Urea-^{13}C, urea-^{12}C, and urea-^{13}C-$^{15}N_2$ have been prepared in partial molar quantities by this method. The triply labeled uurea has been converted by treatment with methyl p-toluenesulfonate ($TsOCH_3$) (7) to the tosylate salt of O-methylisourea-^{13}C-$^{15}N_2$ which has been incorporated into the guanido portion of L-arginine by reaction with the copper complex of L-ornithine (8).

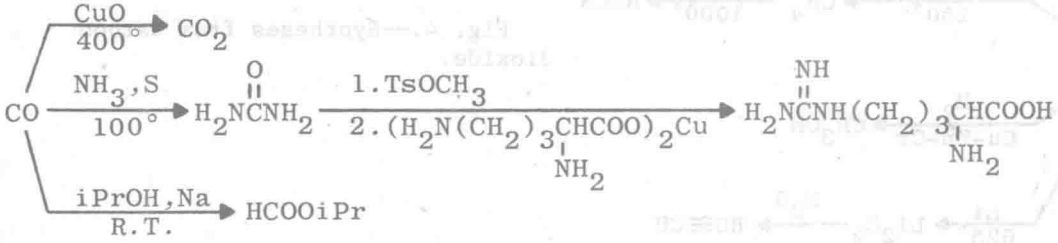

Fig. 2.--Syntheses from carbon monoxide.

Syntheses from Isopropyl Formate (Fig. 3):--Treatment of isopropyl formate with ammonia yields formamide which has been dehydrated to sodium cyanide by an adaptation of the nitrile synthesis of Yamato and Sugasawa (9). This method seems promising for preparation of cyanide labeled with carbon and/or nitrogen. Isopropyl formate has been used to incorporate isotopic carbon into cyclopentanol by condensation with the di-Grignard reagent of 1,4-dibromobutane. This synthesis is an improvement in one of the methods (10) available for preparing isotopic cyclopentadiene. Isopropyl formate-^{13}C has also been the source of carbon-13 in our current work on tryptophan-2-^{13}C (8). N-Formyl-^{13}C o-toluidine, which has been prepared from isopropyl formate and the Grignard reagent of o-toluidine, has been cyclized to indole-2-^{13}C which undergoes a Mannich aminomethylation to yield gramine-2-^{13}C. The subsequent alkylation and hydrolysis reactions currently are being investigated.

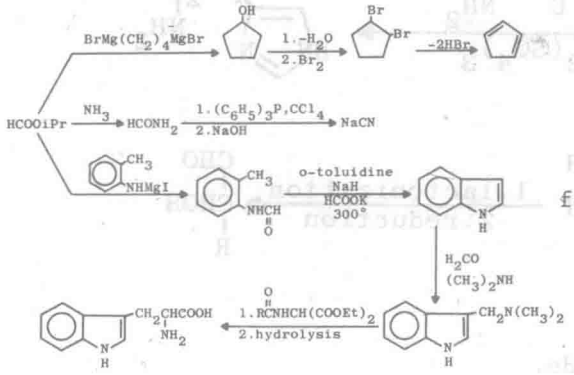

Fig. 3.--Syntheses from isopropyl formate.

Syntheses from Carbon Dioxide (Fig. 4):--In addition to carboxyl-labeled carboxylic acids available by carbonylation of the appropriate Grignard reagent, a number of intermediate compounds can be derived from carbon dioxide. Methane, which has been prepared by hydrogenation of carbon dioxide over a ruthenium catalyst (11), has been converted to sodium cyanide by passing a mixture of methane and ammonia through a tube containing a platinum catalyst and heated to 1000° (12). This reaction currently is used for preparing mole quantities of cyanide but cannot conveniently be used for nitrogen labeling, since it employs a large excess of ammonia. Our multimolar preparations of methanol (both carbon-13 and carbon-12) are carried out by hydrogenating carbon dioxide with a copper-zinc-chromium catalyst (13) in a suitable apparatus. A smaller scale apparatus, constructed from standard laboratory pressure equipment, also has been used for this purpose (5). Acetylene (with carbon-13 or carbon-12) has been prepared by allowing carbon dioxide to react with molten lithium at 625° followed by subsequent hydrolysis of the resulting lithium carbide (14).

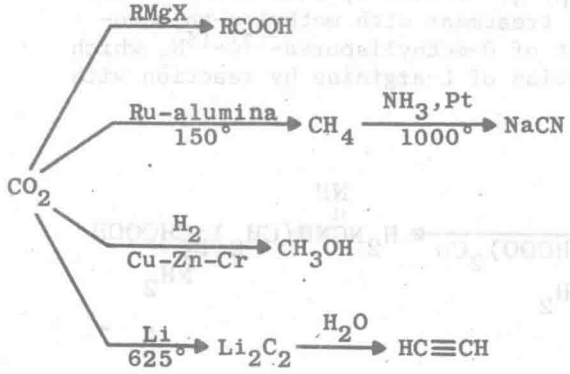

Fig. 4.--Syntheses from carbon dioxide.

Syntheses from Sodium Cyanide (Fig. 5):--Cyanide is a versatile intermediate which can be used to incorporate isotopic carbon into a variety of molecules. Our primary utilization of sodium cyanide has been the preparation of carbohydrates labeled with carbon-13 at C-1 by the Kiliani-Fischer chain lengthening synthesis. Thus, we have prepared glucose-1-^{13}C (and the concomitant mannose-1-^{13}C) from arabinose (15). We are currently working on the synthesis of lactose-1-^{13}C by the same method. Sodium cyanide-^{13}C also has been incorporated into the 2 position of histidine (8) by a short series of reactions beginning with conversion of sodium cyanide to sodium thiocyanate. Condensation of the thiocyanate with L-γ-ketoornithine (obtained from the partial degradation of L-histidine) produced L-2-mercaptohistidine-2-^{13}C which was converted to L-histidine-2-^{13}C by treatment with ferric sulfate.

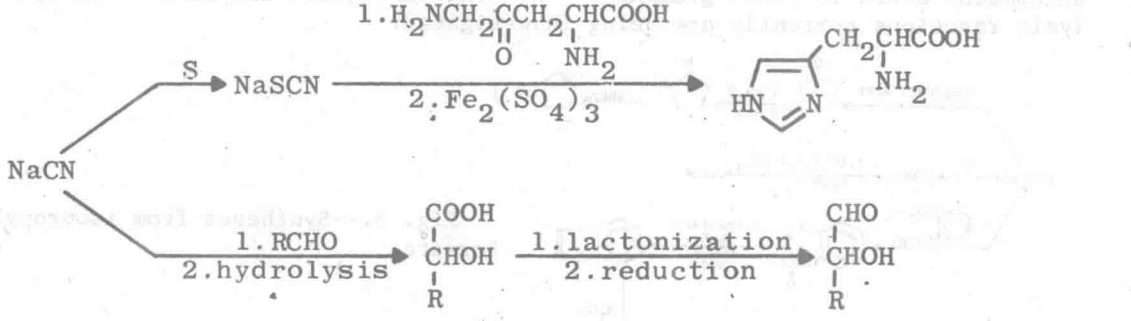

Fig. 5.--Syntheses from sodium cyanide.

Syntheses from Methanol (Fig. 6):--The major use of methanol has been preparation of acetic acid by carbonylation with carbon monoxide in the presence of rhodium trichloride and hydriodic acid (16). This reaction has allowed the synthesis of all possible isotope isomers of acetic acid from the proper combinations of methanol and carbon monoxide. Cysteine-3-^{13}C has been prepared by condensation of diethyl N-dithiocarbobenzyloxyaminomalonate (17) and formaldehyde-^{13}C which, in turn, was obtained by vapor phase oxidation of methanol-^{13}C over an iron-molybdenum-manganese (18) catalyst. Methyl-^{13}C iodide, a useful alkylating agent, has been synthesized from methanol and phosphorus triiodide (19). Methyl iodide has been converted to methylamine by a Gabriel synthesis, and the methylamine subsequently has been alkylated to dimethylamine via the p-toluenesulfonamide (20). This reaction sequence has resulted in the synthesis of dimethyl-$^{13}C_2$-$^{2}H_6$-amine-^{15}N which is being utilized in preparation of a fully isotopic compound (21).

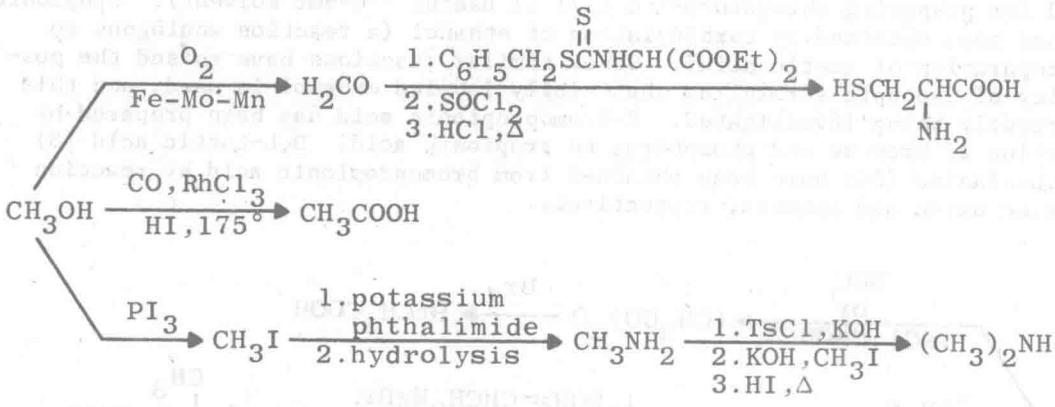

Fig. 6.--Syntheses from methanol.

Syntheses from Acetylene (Fig. 7):--Acetylene (doubly labeled with either carbon-13 or carbon-12) has proven to be a valuable intermediate for preparation of multiply labeled compounds. The ambient temperature trimerization of acetylene on a vanadium oxide-alumina catalyst (22) has been used to prepare uniformly labeled benzene. The stable ethylenediamine complex of lithium acetylide has been synthesized by allowing acetylene to react with N-lithioethylenediamine (23). Some of our current work with acetylene involves the hydration of acetylene to acetaldehyde which can be converted to lactic-2,3-$^{13}C_2$ acid via the cyanohydrin reaction (8) or to alanine-2,3-$^{13}C_2$ by the Strecker amino acid synthesis (24).

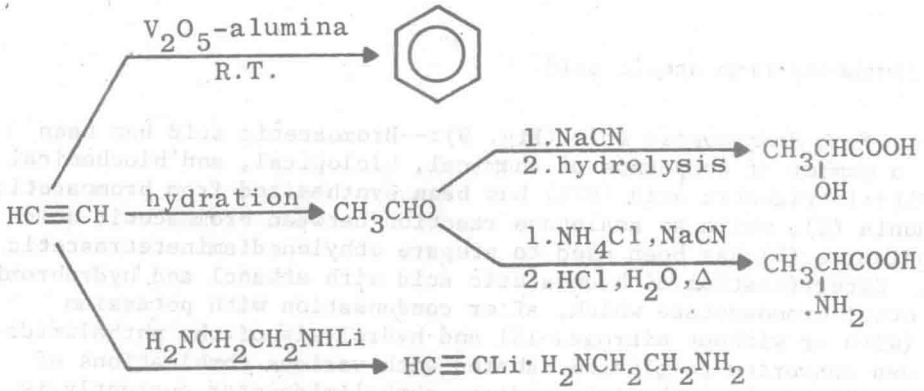

Fig. 7.--Syntheses from acetylene.

Syntheses from Acetic Acid (Fig. 8):--A large variety of labeled compounds can be prepared from acetic acid, and numerous labeling combinations are possible owing to the availability of the isotope isomers of acetic acid itself. Acetic anhydride has been prepared by dehydrating acetic acid with dicyclohexylcarbodiimide (DCC) (25) or by the action of p-toluenesulfonyl chloride (TsCl) on sodium acetate (8). In addition to being a potential acylating agent, acetic anhydride has been converted to bromoacetic acid by direct bromination (8). A number of standard methods can be used to esterify acetic acid. One current synthesis involving methyl acetate is the preparation of 3-hydroxy-3-methylglutaric acid by oxidation of the carbinol obtained from the reaction of allylmagnesium bromide with methyl acetate. Ethanol has been prepared by the rhenium catalyzed hydrogenation of acetic acid (26). We have found that this reaction can be carried out at hydrogen pressures of 1000 psi over a period of several days. Hypochlorite oxidation of ethanol-2-^{12}C has been studied as a method for preparing chloroform-^{12}C (27) (a useful ^{13}C-nmr solvent). Propionic acid has been obtained by carbonylation of ethanol (a reaction analogous to the preparation of acetic acid). Other similar reactions have raised the possibility of isotopic scrambling when singly labeled ethanol is used, and this is currently being investigated. 2-Bromopropionic acid has been prepared by the action of bromine and phosphorus on propionic acid. D,L-Lactic acid (8) and D,L-alanine (24) have been obtained from bromopropionic acid by reaction with zinc oxide and ammonia, respectively.

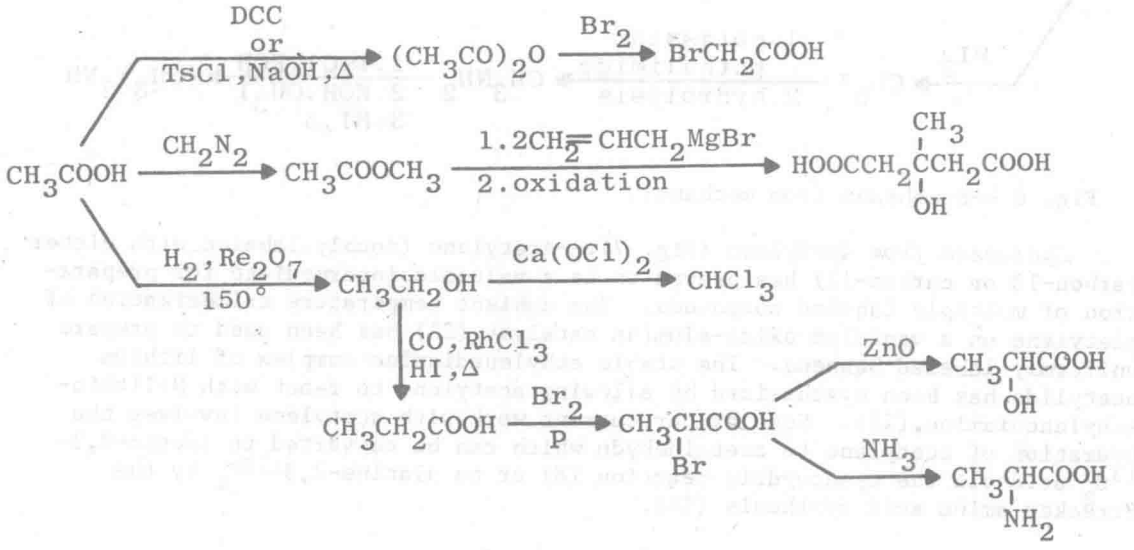

Fig. 8.--Syntheses from acetic acid.

Syntheses from Bromoacetic Acid (Fig. 9):--Bromoacetic acid has been converted to a number of compounds of chemical, biological, and biochemical interest. Nitrilotriacetic acid (NTA) has been synthesized from bromoacetic acid and ammonia (8), while an analogous reaction between bromoacetic acid and ethylenediamine (8) has been used to prepare ethylenediaminetetraacetic acid (EDTA). Esterification of bromoacetic acid with ethanol and hydrobromic acid yields ethyl bromoacetate which, after condensation with potassium phthalimide (with or without nitrogen-15) and hydrolysis of the phthalimidoester, has been converted to glycine labeled with various combinations of carbon-13 and nitrogen-15. The intermediate phthalimidoester currently is being investigated as a precursor to δ-aminolevulinic acid. The amino group of glycine has been methylated with dimethylsulfate (8) in order to prepare carboxymethyltrimethylammonium chloride and the corresponding ester.