

data. If the quality of the laboratory is poor then the decision may be made to ignore the laboratory result and proceed to "care for the clinical condition of the patient." Alternately, the physician may decide to reorder the test a second time to see if a statistically different result is obtained.

Most interferences in analytical methodologies can be eliminated by a chromatographic sample preparation step prior to analysis. However, chromatographic separations are not usually automated, are poorly mechanized, and add time and expense to the analysis. In a high volume service clinical chemistry laboratory, the extra step required by a chromatographic separation is very undesirable. Therefore, today many inaccuracies remain with the routine methods used rather than adapt a cure for the accuracy problem by adding a manual chromatographic sample preparation step.

In conclusion, the development of highly accurate instrumental methods of chemical analysis for the clinical laboratory remains a very difficult task. When one includes the nonanalytical priorities in method development, then decreasing emphasis on accuracy is often accepted by members of this field. Decentralization of the service laboratory causes some additional reassessment of the criteria for test methods. A list of desired characteristics for test methodology can be created. That list of performance criteria in decreasing order of importance is: 24-hour availability of results, minimum sample pretreatment, wide selection of different types of tests, low operator skill, high precision, low total cost per analysis, short throughput time, and high accuracy.

Analytical chemistry has much to contribute to the field of clinical chemistry analysis but ways must be found to produce highly accurate test results while lowering the cost and decreasing the difficulty of the analysis. We as analytical chemists have addressed the cost issue but have not contributed much to the improvement of accuracy while simplifying the technical difficulty of the clinical laboratory tests. This last task then remains as a challenge for the near future.

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Technical Difficulties Associated with the Formation of Carbon-11 Labelled Carboxylic Acids

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Positron Emission Tomography (PET) is an established method for probing biochemical processes in vivo and holds promise for being an established clinical tool [1-4]. PET produces cross-sectional tomographic images which monitor fundamental physiologic, biochemical and pharmacological processes such as glucose metabolism, oxygen utilization, blood flow, amino acid and fatty acid transport and metabolism, tissue receptor and drug binding characteristics. PET is similar to x-ray Computed Tomography (CT) and Magnetic Resonance Imaging (MRI) in that it is a computed tomographic technique. Unlike these clinical techniques, PET requires the injection of a positron emitting compound of particular physiologic interest. At present, the positron emitting radionuclides commonly being investigated include carbon-11 ($t_{1/2}=20.4$ m), nitrogen-13 ($t_{1/2}=9.98$ m), oxygen-15 ($t_{1/2}=123$ s) and fluorine-18 ($t_{1/2}=109.8$ m). The restrictions imposed upon the pharmaceutical chemist due to the physical half lives of these radionuclides mandate not only a sensitive, but also a rapid quality assurance program for the finished formulations. The preparation of a class of fatty acids is an excellent illustrative example of the technical difficulties and chemical subtleties associated with the research effort on short-lived radiopharmaceutical diagnostic compounds.

Biologically, long chain fatty acids are important substrates utilized to meet the myocardial energy requirements. It is theorized that PET might provide insight into changes in the utilization of these compounds as a result of therapies or organ

dysfunction caused by disease. Moreover, an added benefit of the successful synthesis of particular fatty acids is that it allows the radiopharmaceutical chemist the option of preparing other synthons, such as acid chlorides, aldehydes and alcohols.

Fatty acids labelled with radio-carbon are prepared from the corresponding organomagnesium halide. Although numerous fatty acids have been prepared utilizing this synthetic approach, few detailed reports exist. For this reason we now report our results on the preparation of (^{11}C)-cyclopropanecarboxylic acid and (^{11}C)-3,4-dimethoxybenzoic acid. These compounds are required as starting reagents for the preparation of (^{11}C)-cyclofoxy [1,2] and (^{11}C)-dopa [3,4] respectively.

Experimental

"No carrier added" (^{11}C)-carbon dioxide is produced in high radionuclidic and radiochemical purity by the $^{14}\text{N}(\text{p}, \alpha)^{11}\text{C}$ nuclear reaction on a nitrogen gas target using the Japan Steel Works Limited Baby Cyclotron (BC-1710). The $^{11}\text{CO}_2$ is condensed from the nitrogen gas target stream on a vacuum line using a glass radiator trap cooled to -193°C . Following complete removal of the nitrogen gas, the $^{11}\text{CO}_2$ is allowed to react with freshly prepared organomagnesium halide. After 2-3 minutes, the reaction is quenched by acid hydrolysis. In the experiments involving the addition of carrier, known quantities of carbon dioxide were added to the cyclotron produced product.

A catalytic concentration of 1,2 dibromoethane was used to initiate the reactions [6]. The concentration of the alkyl magnesium halide formed, with suitable correction made for non-Grignard basicity [5], was determined by acidimetric titration using CCl_4 . Moreover, radio thin layer chromatography was used to establish the radiochemical purity of the synthesized fatty acid.

The chemical scheme and specific reactions leading to the products are shown in figure 1 and our analytical results are summarized in table 1.

Discussion

The reaction of Grignard reagents with radiolabelled carbon dioxide has proven to be an effective means of preparing labelled carbonyl compounds. The state of the magnesium surface has a significant impact upon the yield of the reaction

and subsequent "side" reactions. It is important to use high chemical purity magnesium with a large surface area for the preparation of the organomagnesium reagent. The principal competing reactions that lead to a reduced yield of Grignard reagent are a) a coupling (Wurtz) reaction and b) a disproportionation reaction. These reactions are favored by elevated temperatures and excess halide concentration. Our experience indicates that the formation of the Grignard reagent proceeded smoothly when the alkyl bromide concentration was greater than 0.15 mol/L. The freshly prepared Grignard reagent exhibits a high chemical reactivity. Efforts to exclude oxygen, moisture and stable carbon dioxide are important to maintain the high specific activity and to minimize "side" reactions. Only freshly distilled solvents, flame-dried magnesium turnings and glassware, and an inert atmosphere are appropriate.

The radio thin layer chromatography shows that several radioactive products are formed which lead to lower chemical yield of the labelled fatty acid. These products could be the result of competing reactions resulting in ketone formation or alcohol formation from impure or excess Grignard reagent. In the preparation of "no carrier added" radiopharmaceuticals side product formation is generally limited by restricting reaction times.

In conclusion, the preparation of "no carrier added" carbon-11 labelled fatty acids is readily achievable utilizing organomagnesium compounds and carbon dioxide labelled with carbon-11; however, impure reagents and experimental conditions may reduce the concentration of the desired Grignard reagent. To find application in PET, the preparation of both radionuclidic and radiochemically pure compounds is of paramount concern. In the case of radiolabelled carboxylic acids, care must be exercised in determining the yield of Grignard reagent formation based solely upon assay of nonisolated intermediates.

Accuracy in Trace Analysis

Table 1. Formation yields of magnesium compounds and the corresponding carboxylic acids.

	Grignard reagent			Carboxylic acid	
	R'X	Ar-MgBr/Ar-Br	cycloPr-MgBr/cycloPr-Br	Ar-COOH	cycloPr-COOH
Experimental	-	59 (32-100)*	71 (52-100)		
Yield	BrCH ₂ -CH ₂ Br	40 (7-76)	41 (8-71)	42 (25-60)	52 (10-66)
	CCl ₄	-	55 (42-66)		
Published		-	80	95<	60-80
Yield					

* The numbers in parentheses represent the extremes in our experimental results.

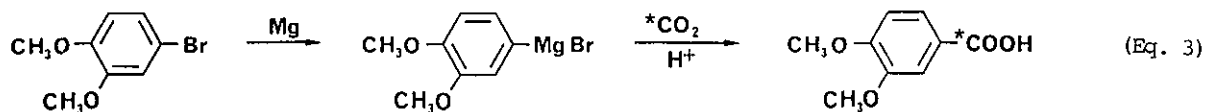
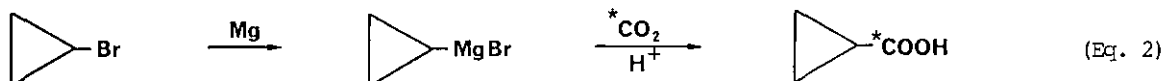
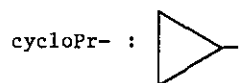
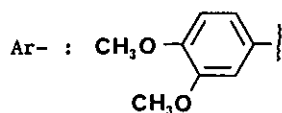


Figure 1. Activation of magnesium by entrainment eq (1) and reactions leading to the formation of radiolabelled cyclopropanecarboxylic acid eq (2) and 3,4-dimethoxybenzoic acid eq (3).

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The Development of Definitive Methods for Organic Serum Constituents

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Methods in clinical chemistry must be fast, cost-effective, and relatively easy to perform. But, of more importance, they must be sufficiently accurate and precise for proper interpretation of the results by physicians. For the most common serum analytes, a variety of methods and instrumentation are used for each. These methods vary in performance and generally exhibit a bias versus each other. An accuracy base is needed to provide a means of evaluating reference and field methods and the performance of the laboratories using them. The concept of definitive methods was developed to provide such an accuracy base. The mechanism for this accuracy transfer is through serum pools with concentrations certified by definitive methods.

For a method to be considered definitive, it must be based upon sound theoretical principles, its accuracy must be tied to an absolute physical quantity, it must be thoroughly tested for sources of bias and imprecision, and it must produce results which approximate the "true value" within narrow limits.

In cooperation with the College of American Pathologists (CAP), the National Bureau of Stan-

dards (NBS) has a long-running program on development of definitive methods. Isotope dilution mass spectrometry (IDMS) is the technique of choice for definitive methods and has been used for many serum analytes, both organic and inorganic. The organic analytes for which candidate DM's have been developed at NBS include cholesterol [1], glucose [2], urea [3], uric acid [4], and creatinine [5]. These methods have been used to certify analyte concentrations in a freeze-dried human serum Standard Reference Material as well as a number of frozen and freeze-dried serum pools supplied by the Centers for Disease Control, the College of American Pathologists, and others.

Although the individual methods vary, they have several common features. All involve addition of a weighed amount of a stable isotope labeled analogue of the compound of interest to a weighed amount of serum, followed by an equilibration period. The labeled and unlabeled forms are isolated from most of the matrix, derivatized, and subjected to GC/MS measurement of the intensity ratio of an ion from the unlabeled analyte and the corresponding ion from the labeled compound. This intensity ratio is compared with intensity ratios of standard mixtures measured immediately before and after the sample measurement. With this approach relative standard deviations for independent preparations have ranged from 0.2–0.4%. Measurements made using other pairs of ions and different gas chromatography conditions are employed to assure the absence of bias in the measurements. The labeled analogue, the derivative, and the ions monitored for each analyte are listed in table 1.

Table 1. Labeled analogues, derivatives, and principal ions monitored for each analyte

Analyte	Labeled analogue	Derivative	Ions measured
Cholesterol	cholesterol-d ₇	TMS ether	458,465
	cholesterol- ¹³ C ₃	"	458,461
Glucose (2 methods)	glucose- ¹³ C ₆	diacetone	245,250
	"	butylboronate-acetate	297,302
Urea	urea- ¹⁸ O	6-methyluracil-TMS	255,257
Uric Acid (2 methods)	uric acid- ¹⁵ N ₂	tetraethyl	280,282
	"	t-butylidimethylsilyl	567,569
Creatinine	creatinine- ¹³ C ₂	ethyl ester of N-(4,6-dimethyl-2-pyrimidinyl)-N-methylglycine	150,152