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CONVENIENT LABELLING TECHNIQUE FOR MASS SPECTROMETRY: ACID CATALYZED DEUTERIUM AND OXYGEN-18 EXCHANGE VIA GAS-LIQUID CHROMATOGRAPHY

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The technique of prime importance in the elucidation of electron-impact-induced fragmentation mechanisms and rearrangement processes in an organic molecule is the selective substitution of deuterium for hydrogen at exchangeable sites. The preparation of oxygen-18 labelled compounds is of additional value in providing complementary mechanistic information. A recent review (1) concerning the exchange of enolizable hydrogen for deuterium by "wet" chemical means emphasizes the wide variety of conditions (solvent, catalyst, temperature, time, purification, etc.) employed routinely to effect labelling of milligram quantities of sample with high isotopic purity. Recently the exchange of enolizable hydrogen for deuterium by a single passage of sample through a predeuterated alkaline GLC (gas-liquid chromatographic) column has been reported (2). The average isotopic purity of the resulting labelled ketones was shown to be 96% by mass spectrometric analysis; whereas, amides, aldehydes and esters

displayed less satisfactory results.

Current results employing acidic exchange conditions on a tandem GLC column (3) have extended the scope of applicability of the GLC labelling method in several major aspects. Ketones which incorporated an average of 96% deuterium under alkaline conditions (2), exchanged with analogous ease under the prescribed acidic conditions. The compounds listed in Table 1 were chromatographed at the physical parameters indicated, in each case a single passage of sample through the column was performed, and the collected sample analyzed mass spectrometrically (4). Carbonyl compounds which were precluded previously by the nature of additional acidic functionality, e.g. carboxyl and phenolic hydroxyl groups, showed essentially quantitative uptake of deuterium under acidic conditions. Also the exchange of polyfunctional compounds containing easily saponifiable groups and/or alkali reactive functions, e.g. keto esters and aldehydes, respectively, were labelled satisfactorily as indicated in Table 1.

In addition to the augmented scope for deuterium labelling, the acidic medium has allowed extension of this method to oxygen-18 labelling for the compounds discussed above, see Table 1 for comparison of deuterium and oxygen-18 incorporation. A further point to be mentioned concerns molecules which undergo reaction under acidic conditions to yield acidic functional groups, e.g. acid chlorides and anhydrides, thereby incorporating oxygen-18 in the process. As demonstrated by the O-18 data in Table 1, the effectiveness of the oxygen labelling is quite satisfactory.

TABLE 1

Labelled Compound	Deuteration Data		Oxygen-18 Data[a]	
	% Total Exchange	GLC Conditions[b]	% Total Exchange	GLC Conditions[b]
methyl-n-nonyl ketone	96-(d_5)	180/5	40	180/4
3-tetradecanone	96-(d_4)	180/14	43	178/15
valerophenone	90-(d_2)	200/7	45	180/7
isovalerophenone	86-(d_2)	200/6	51	180/5
caprophenone	90-(d_2)	180/9	49	178/11
alpha-naphthylmethyl-ketone	92-(d_3)	200/11	51	225/3
beta-naphthylmethyl-ketone	-	-	49	220/4
n-butyl levulinate	89-(d_5)	125/20	39	180/4
o-hydroxyacetophenone	-	-	45.5	180/3
m-nitroacetophenone	88-(d_3)	180/11	50	180/11
n-nonylaldehyde dimer	89-(d_2)	190/19	60	190/19
n-tetradecaldehyde	80-(d_2)	190/5	55	160/16
o-tolualdehyde	-	-	65	152/3
p-tolualdehyde	-	-	60	160/3
benzoic acid[c]	-	-	29[d]	160/2
beta-naphthoic acid[c]	-	-	67	208/3
acetic acid[e]	-	-	31	162/1

a All compounds were labelled with 71% enriched H_2O^{18} (Weizmann Institute).

b Column temperature (°C)/retention time listed in column; carrier gas flow rate was 40-50 ml./min.

c Obtained from the corresponding acid chlorides via hydrolysis on the column.

d 31% enriched H_2O^{18} was used.

e Obtained from column hydration of acetic anhydride.

It should be noted that this tandem column arrangement preserves all purification advantages of the GLC labelling approach (2), by allowing separation on a wide selection of proper liquid phases, i.e. phases which are otherwise incompatible with phosphoric acid at elevated temperatures.

The ease and rapidity with which exchange of enolizable hydrogens and carbonyl oxygens can be quantitatively achieved, emphasizes the practical utility of the tandem GLC column. The method is well suited for the high quality preparation of pure labelled samples of synthetic and natural products available in only minute amounts.

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REFERENCES

1. For a comprehensive review of deuteration procedures, see: H. Budzikiewicz, C. Djerassi and D. H. Williams, Structure Elucidation of Natural Products by Mass Spectrometry, Volume I: Alkaloids, Holden-Day Inc., San Francisco, 1964, Chapter 2.

2. M. Senn, W. J. Richter and A. L. Burlingame, J. Am. Chem. Soc., 87, 680 (1965).

3. The tandem GLC column consisted of two 1/4" x 5' coiled stainless steel columns linked with a Swagelock union. The column packing in the first section (exchanger) consisted of Chromosorb W (45/60 mesh) coated with 5% phosphoric acid; in the second section (separator), a liquid phase was selected depending on the chromatographic parameters required for the type of compound, e.g. SE-30, Apiezon L, Carbowax 20M, etc. Preparation of this tandem column for use required: (1) for deuteration, conditioning by injection of approximately 300 microliters of D_2O and stabilization for 5-10 hours; (2) for oxygen-18 exchange, sequential injection of H_2O^{18} followed by the sample within 10 seconds. In general, 4 mg. H_2O^{18} were used per milligram of compound.

4. All mass spectra were determined on a modified C.E.C. 21-103C mass spectrometer (for details of modifications and performance, see F. C. Walls and A. L. Burlingame, Anal. Chem., in preparation) equipped with a heated glass inlet system operated at 200°C. Most spectra were recorded at ionizing voltage 70 e.v., ionizing current 10-50 μa and 160-180 volts per stage on the multiplier. Representative samples were determined employing a direct inlet system (see, A. L. Burlingame, in Advances In Mass Spectrometry, Vol. III, Pergamon Press, in preparation) and scanning the entire spectrum in 30 seconds.