Tetrahedron Letters No.13, pp. 1223-1226, 1967. Pergamon Press Ltd. Printed in Great Britain.

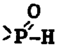
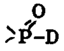
THE USE OF PREPARATIVE GAS-LIQUID CHROMATOGRAPHY FOR DEUTERIUM LABELLING OF ORGANIC COMPOUNDS

G. J. Kallos and L. B. Westover

Chemical Physics Research Laboratory
The Dow Chemical Company
Midland, Michigan

(Received 5 December 1966; in revised form 23 January 1967)

The assignment of certain bands in infrared spectra and the elucidation of electron-impact-induced fragmentation mechanisms of organic molecules often require the substitution of a specific hydrogen such as OH or NH with deuterium. A comprehensive review (1) regarding the exchange of hydrogen with deuterium emphasizes the use of various "wet" chemical methods in order to obtain small quantities of labeled sample with maximum purity. The quantitative exchange of enolizable hydrogen atoms for deuterium atoms in ketones by a single pass of sample through an analytical gas-liquid chromatographic column pretreated with deuterium oxide has been reported recently (2). The deuterium exchange for active hydrogens has been accomplished as well by employing acidic conditions (3). Although small amounts of deuterated sample may be used for mass spectrometric studies, the need for Infrared and Raman Spectroscopy require much greater sample for band assignments. The present work was undertaken to test the general application of deuterium labelling via preparative scale gas-liquid chromatography.

It has been found that an almost quantitative exchange (>90% total deuterium exchange) of  $>\overset{\overset{O}{/\!/}}{P}-H$ to  $>\overset{\overset{O}{/\!/}}{P}-D$ in O,O-dimethyl hydrogen phosphonate and O,O-diethyl hydrogen phosphonate, -NH to -ND in pyrrole and -OH to -OD in aliphatic alcohols can be accomplished during a single pass on a preparative scale gas chromatograph using a column pretreated with deuterium oxide. The deuterium labelling of the phosphorous compounds by any "wet" chemical procedures would have been difficult, since compounds of this type generally hydrolyze in water. It is interesting to note that 1.5-3.0 gms of the exchanged compounds were collected during a single pass. As shown in Table 1, all ketones will exchange enolizable hydrogens under basic conditions, while a keto-alcohol will exchange only the -OH proton under normal conditions. Any attempts to exchange enolizable hydrogen atoms in ketones without KOH did not produce any exchange.

The chromatographic units used in this work were built in this laboratory by L. B. Westover and R. S. Gohlke. The large unit consists of a cylindrical glass tube six feet long and one inch inside diameter. The small unit contained a straight stainless steel tube three feet long and 1/2 inch inside diameter. The column packing consisted in most cases of chromosorb ω (30-60 mesh) coated with 25% carbowax 4000 or 25M. Thermal conductivity detectors were used and helium as the carrier gas. The helium was dried free of moisture.

The preconditioning of the column was carried out be passing a total of 6.8 cc. of D_2O with approximately 1.8 cc. injection at 3-5 minutes interval. After all water had eluted through the column, the sample was injected (1.6-3 cc.) and collected in a preconditioned trap.[a] All compounds listed in Table 1 were exchanged under the conditions prescribed, and the collected sample analyzed by Infrared and NMR spectroscopy (4).

The isotopic purity of a deuterated compound was found to be improved by making a second pass through the column after it has been retreated with D_2O. The amount of D_2O used to retreat the column was varied with column size, amount of packing and substrate. Although originally 6.8 cc. of D_2O was needed to condition a carbowax E-4000 column in the large unit, 3-4 cc. of D_2O was necessary to recondition for successive passes. It has been experienced that the conditioned column lost a major portion of deuterium, when left overnight with helium flowing through it.

It should be noted that this technique could serve a dual role in that a sample can be deuterated and separated from its impurities at the same time. For example, a small amount of methanol impurities eluted faster than O,O-dimethyl hydrogen phosphonate under the conditions described.

---

a. Trap was rinsed with small amount of D_2O and dried in an oven at 110°C for a few minutes.

Table 1

Labelled Compound	% Deuterium Exchange (Single Pass)	GLC Conditions[b]	Column Packing
O,O-dimethyl deuterium phosphonate	>90	95/58/1150	A
O,O-diethyl deuterium phosphonate	>90	95/50/2000	A
Ethanol-OD[c]	>90	75/24/85	A
Sec-butanol-OD	>92	95/5/1150	A
Pyrrole-ND[c]	>93	98/13/200	A
1-heptanol-OD	86	137/13/1150	B
1-hydroxy-3-methyl-2-butanol[e]-OD	66	139/30/1150	B
Acetophenone-d_3	85	152/18/1150	C
4-methyl-2-pentanone-d_5	36[d]	85/15/500	C
4-Decanone-d_4	90	137/16/1150	C
2-hexanone-d_5	62	114/5/1150	C
Valeraldehyde[f]	0	102/3/1150	B

A - 25% carbowax 4000 B - 25% carbowax 20M

C - 15% carbowax 20M + 10% KOH

The quantitative exchange and high quality preparation of deuterated samples through preparative gas-liquid chromatography render this technique very valuable for obtaining large amounts of sample for Infrared and Raman Spectroscopic studies in a short period of time.

---

[b] Column temperature (°C)/retention time (min.)/carrier gas flow rate (ml./nim.)

[c] Small unit. The amount of D_2O used in preconditioning of column and sample injected was reduced by a factor of five from that employed in large unit.

[d] Obtained 70% total exchange by making three passes.

[e] Produced mainly methyl isopropenyl ketone (90% exchange of enolizable hydrogens) with Column packing C.

[f] Reacted with Column packing C.

Acknowledgment

The generous help of W. F. Scott and D. C. Charboneau for their experimental assistance, and R. A. Nyquist, R. O. Kagel, and J. P. Heeschen for analyzing the samples, is greatfully acknowledged.

References

1. H. Budzikiewiez, C. Djerassi, and D. H. Williams, *Structure Elucidation of Natural Products by Mass Spectrometry*, Volume 1: *Alkaloids*, *Holden-Day*, *In.*, San Francisco, 1964, Chapter 2.
2. M. Senn, W. J. Richter, and A. L. Burlingame, *J. Am. Chem. Soc.*, **87**, 680 (1965).
3. W. J. Richter, M. Senn, and A. L. Burlingame, *Tetrahedron Letters*, **17**, 1235 (1956).
4. All Infrared and NMR spectra were determined on a Beckman IR-9 and Varian A-60, respectively.