

Short Communication

Hydrogen–deuterium exchange in fused-silica capillary columns

MOHAMED E. MAHMOUD

Barnett Institute of Chemical Analysis and Department of Chemistry, Northeastern University, Boston, MA 02115 (USA)

ADEL M. MOUSSA

Barnett Institute of Chemical Analysis, Northeastern University, Boston, MA 02115 (USA)

DAVID A. FORSYTH

Department of Chemistry, Northeastern University, Boston, MA 02115 (USA)

and

PAUL VOUROIS*

Barnett Institute of Chemical Analysis and Department of Chemistry, Northeastern University, Boston, MA 02115 (USA)

(First received January 9th, 1991; revised manuscript received March 13th, 1991)

ABSTRACT

Ketones deuteriated α to the carbonyl have been observed to undergo significant isotope exchange on fused-silica capillary columns during gas chromatography–mass spectrometry. The residence time of the compounds on the column was found to influence the extent of isotope exchange. The degree of exchange was examined using a variety of columns and the isotope exchange was found to occur even with brand new columns. Conversion of the keto compounds into the methyloxime derivatives resulted in retention of the “correct” isotope content during gas chromatography–mass spectrometry.

INTRODUCTION

Hydrogens α to a carbonyl are well known for their activity and this property is exploited to introduce deuterium atoms in that position via exchange under acidic or basic conditions [1]. The preparation of labeled compounds for use as internal standards in quantitative analysis by gas chromatography–mass spectrometry (GC–MS) often involves the use of appropriate keto intermediates to facilitate incorporation of the label(s) in the desired molecule.

In packed column GC–MS, chromatographic columns have been used to provide a source of isotopic label for the exchange of labile hydrogens (or entire function-

al groups) on-line in order to assist in the interpretation of spectra. For example, hydroxylic or amino hydrogens can be readily replaced with deuterium by prior saturation of the column's active sites with $\text{CH}_3\text{O}^2\text{H}$ [2,3]. Columns impregnated with $\text{Ba}(\text{O}^2\text{H})_2$ have been used on-line to exchange hydrogen α to carbonyls in alicyclic systems [4]. Isotopic labeling using different sources of isotopes have been reported [5-8]. Moreover, the higher acidity of trimethylsilyl (TMS) esters of TMS-phosphates has been taken advantage of in order to effect selective exchange of the former groups in packed columns previously saturated with $[\text{}^2\text{H}_9]$ bis-trimethylsilyl acetamide [9,10].

In recent experiments we observed that fused-silica capillary columns exhibit sufficient activity to cause exchange of hydrogens α to a carbonyl. We initially encountered this *a priori* unexpected tendency for ^1H - ^2H exchange while working with capillary columns that had been previously used for general analytical purposes. In this paper we report a more detailed examination of the phenomenon using both "old" and "new" fused-silica capillary columns from different manufacturers. The results of these studies are presented below.

EXPERIMENTAL

Instrumentation

GC-MS studies were conducted with a Finnigan 4021B mass spectrometer interfaced to a Hewlett-Packard 5890 gas chromatograph (equipped with on-column injector) and Incos data system. The mass spectrometer was operated in the electron impact (EI) mode over a limited mass range so as to record the molecular ion region of each compound of interest. The ion source temperature was set at 250°C and the ionizing energy at 70 eV. The unlabeled counterparts of all compounds were examined in order to establish the potential contribution from $(M-1)$ and/or $[M+1]$ peaks to the spectra.

Compounds

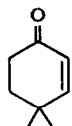
The isotopically labeled compounds used in this study were prepared as follows: 4,4-Dimethyl-2-cyclohexen-1-one, 4-methylcyclohexanone, and 3-keto-androstane were prepared by dissolving 50 mg of the respective compound in 10 ml solution of 40% NaO^2H and $^2\text{H}_2\text{O}$ and stirring the reaction mixture for 3-4 hours at 80°C . The products were then extracted from diethyl ether and dried over Na_2SO_4 .

The correct isotopic ratios for the various compounds were established by analyzing them via the direct insertion probe (DIP) in order to avoid extensive contact of the analytes with active surfaces. To compensate for the high volatility of most of the studied deuteriated compounds, mass spectra were obtained by using a cold probe tip and scanning the mass spectrometer immediately upon opening the valve leading to the high vacuum system and the ion source.

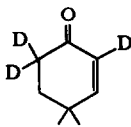
RESULTS AND DISCUSSION

The structures of the ketones used to ascertain the propensity of α hydrogens to undergo exchange, the position of deuterium incorporation and their molecular weights are presented below. Since the percentage of isotopic incorporation in the

I: 4,4-Dimethyl-2-cyclohexene-1-one

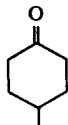


(124)

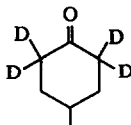


(127)

II: 4-Methylcyclohexanone

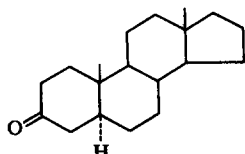


(112)

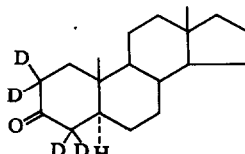


(116)

III: 3-Keto-androstane



(274)



(278)

compounds was less than 100%, the ratio of several "molecular ion" peaks encompassing various degrees of isotopic content from M^{+} to $M^{+} + n_i$ (where n_i = the number of deuterium atoms) had to be monitored.

TABLE I

RATIO OF MOLECULAR ION PEAKS OF $^2\text{H}_3$ -LABELED 4,4-DIMETHYL-2-CYCLOHEXENE-1-ONE INTRODUCED AT DIFFERENT FLOW-RATES WITH NEW AND OLD COLUMNS

Ratio is expressed as a percentage relative to the most intense peak which is assigned a value of 100% in the multiplet of peaks relative to the molecular ion. A = Old 30-m DB1 column (J&W), oven-temperature = 150°C, split injector and variable flow-rates. B = Old 60-m DB1 column (J&W), oven-temperature = 200°C, split injector and variable flow-rates. C = New 30-m DB1 column (J&W), oven-temperature = 150°C, split injector and variable flow-rates. D = New 30-m DB1 column (HP), oven-temperature = 150°C, split injector and variable flow-rates.

<i>m/z</i>	Peak ratio at different flow-rates												Probe run				
	6 ml/min				4 ml/min				2 ml/min					1 ml/min			
	A	B	C	D	A	B	C	D	A	B	C	D		A	B	C	D
124 ($^2\text{H}_0$)	87	31	32	42	100	51	42	42	100	100	57	58	100	100	60	-	35
125 ($^2\text{H}_1$)	100	100	100	97	41	100	100	100	28	67	100	100	24	31	100	-	96
126 ($^2\text{H}_2$)	20	15	93	100	18	18	94	90	15	21	84	81	16	16	73	-	100
127 ($^2\text{H}_3$)	19	15	45	53	12	17	47	41	11	16	40	34	10	11	28	-	61
128 ($^2\text{H}_4$)	11	10	9	8	3	10	7	4	2	6	6	4	2	2	2	-	17

Effect of residence time

The contribution of the column to the exchange process was initially evaluated under isothermal conditions by varying the flow-rate in order to assess the influence of residence time. Two old, but otherwise well-performing columns as well as two new 30-m columns from different manufacturers (HP and J&W Scientific) were evaluated. For 4,4-dimethyl-2-cyclohexan-1-one, the results are summarized in Table I. Listed here are the ratios of the undeuterated ($^2\text{H}_0$: m/z 124) and the corresponding labeled analogues ($^2\text{H}_1$: m/z 125; $^2\text{H}_2$: m/z 126; $^2\text{H}_3$: m/z 127) of the compound. The following general trends are observed:

(1) At lower flow-rates (higher residence time) there is an enhancement of the relative abundance of the m/z 124 ion. Note, for example, the variation in the peak intensity ratio from 87:100:20:19 (6 ml/min) to 100:24:16:10 (1 ml/min) for the $^2\text{H}_0$ - $^2\text{H}_1$ - $^2\text{H}_2$ - $^2\text{H}_3$ species.

(2) Even at the highest flow-rate examined (6 ml/min), used columns exhibit sufficient activity to induce exchange.

(3) New columns, as expected, appear less reactive and the ion mass ratios are more comparable to direct insertion probe data especially at shorter residence times.

(4) At longer residence time, *i.e.*, lower flow-rates, the new columns are also sufficiently active to favor formation of an analog with lower deuterium content.

A plot of the variation in distribution of peak intensities "normalized; total = 100" from the 30-m DB1 old column (J&W) is shown in Fig. 1. The peak intensities from analysis of I via the direct insertion probe are included for comparison. An increase in the peak intensity of the unlabeled compound with lower flow-rate is apparent, indicating increased exchange with longer residence time.

Two other related carbonyl compounds labeled at the α position with deuterium were also examined in order to assess the generality of the phenomenon discussed above. The behavior of these compounds on the "new" columns is summarized in

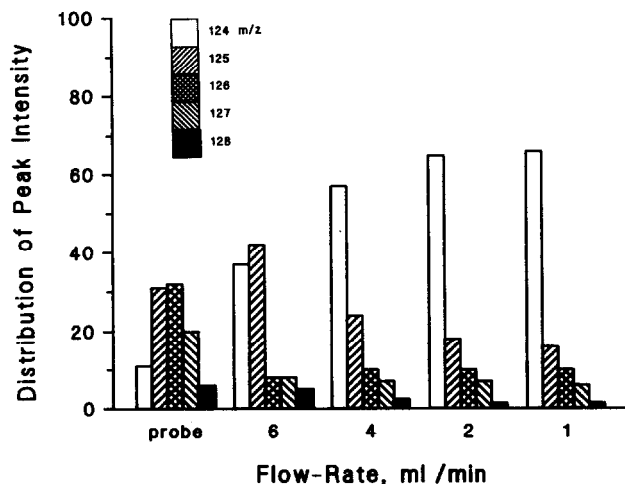


Fig. 1. Variation of the peak intensities "normalized; total = 100" for I from the 30-m DB1 old column (J&W) at different flow-rates.

TABLE II

RATIO OF MOLECULAR ION PEAKS OF $^2\text{H}_4$ -LABELED 4-METHYLCYCLOHEXANONE INTRODUCED AT DIFFERENT FLOW-RATES WITH TWO DIFFERENT NEW COLUMNS

Ratio is expressed as a percentage relative to the most intense peak which is assigned a value of 100% in the multiplet of peaks relative to the molecular ion. A = New 30-m DB1 column (J&W). B = New 30-m DB1 column (HP).

<i>m/z</i>	Peak ratio at different flow-rates						Probe run
	6 ml/min		4 ml/min		1 ml/min		
	A	B	A	B	A	B	
112 ($^2\text{H}_0$)	29	18	33	26	35	25	24
113 ($^2\text{H}_1$)	78	68	100	100	100	80	68
114 ($^2\text{H}_2$)	100	100	98	100	100	100	100
115 ($^2\text{H}_3$)	60	76	54	75	50	61	91
116 ($^2\text{H}_4$)	15	29	18	29	14	21	13

Tables II and III. Again a definite trend is observed that shows increased exchange with longer residence time on the columns. Moreover, a somewhat stronger activity is evident for the (J&W) column as compared to the HP column. Significantly, conversion of compounds II and III to their methyloxime derivatives suppressed any exchange of the α protons. In the latter case, the ratios of peak intensities corresponding to the different molecular ion masses were identical for both the direct insertion probe and GC-MS analyses.

Effect of temperature

In the preceding section we discussed the effect of flow-rate or residence time on the exchange process under isothermal conditions. Clearly temperature is another variable which needs to be considered since, from a practical point of view, GC-MS

TABLE III

RATIO OF MOLECULAR ION PEAKS OF $^2\text{H}_4$ -LABELED 3-KETOANDROSTANE INTRODUCED AT DIFFERENT FLOW-RATES WITH TWO DIFFERENT NEW COLUMNS

Ratio is expressed as a percentage relative to the most intense peak which is assigned a value of 100% in the multiplet of peaks relative to the molecular ion. A = New 30-m DB1 column (J&W). B = New 30-m DB1 column (HP).

<i>m/z</i>	Peak ratio at different flow-rates						Probe run
	6 ml/min		4 ml/min		1 ml/min		
	A	B	A	B	A	B	
274 ($^2\text{H}_0$)	2	0	0	1	2	1	1
275 ($^2\text{H}_1$)	18	3	14	20	62	44	12
276 ($^2\text{H}_2$)	87	43	88	36	100	44	37
277 ($^2\text{H}_3$)	100	100	100	100	78	100	100
278 ($^2\text{H}_4$)	49	96	47	66	19	58	57

TABLE IV

RATIO OF MOLECULAR ION PEAKS OF $^2\text{H}_3$ -LABELLED 4,4-DIMETHYL-2-CYCLOHEXENE-1-ONE AT DIFFERENT ISOTHERMAL ANALYSES AND CONSTANT FLOW-RATE WITH A 30-METER DB1 OLD COLUMN

Ratio is expressed as a percentage relative to the most intense peak which is assigned a value of 100% in the multiplet of peaks relative to the molecular ion.

<i>m/z</i>	Peak ratio at different isothermal analyses				Probe run
	200°C	150°C	120°C	100°C	
124 ($^2\text{H}_0$)	19	22	100	100	35
125 ($^2\text{H}_1$)	100	100	87	33	96
126 ($^2\text{H}_2$)	19	15	23	16	100
127 ($^2\text{H}_3$)	18	15	18	10	61
128 ($^2\text{H}_4$)	11	9	7	1	17

analyses are usually conducted by programming the column temperature. The trends for ^1H - ^2H exchange in I as a function of column temperature were examined. Two sets of experiments were conducted. In the first, the flow-rate was maintained constant at different column temperatures. Table IV summarizes the obtained data which show that more exchange occurs at lower temperature. In the second, the column temperature was ramped at different heating rates. Fig. 2 shows that as the heating rate is decreased, the amount of hydrogen exchange increases significantly. Both of these observations are consistent with more exchange due to longer residence time on the column.

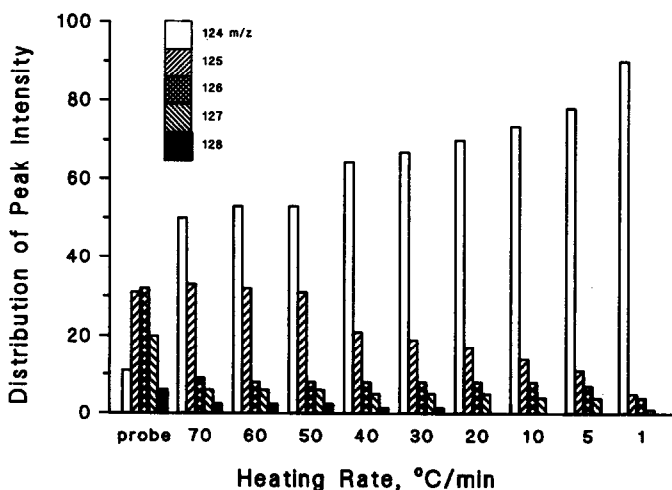


Fig. 2. Variation of the peak intensities "normalized; total = 100" for I from the 30-m DB1 old column (J&W) at different heating rates.

CONCLUSIONS

The data presented above demonstrate that fused-silica capillary columns exhibit sufficient activity to effect exchange of hydrogens α to carbonyls as well. After some use, columns appear to develop a stronger activity towards effecting such an exchange even though they still appear to function well for general analytical purposes. In fact, even the two brand new columns examined here were not inert. In synthesizing isotopically labeled compounds, it is common to test the isotopic purity of intermediates or the final products by capillary GC-MS. While it may be dangerous to generalize from the limited number of examples and columns examined here, it is reasonable to suggest that caution should be exercised in attaching significance to such analyses especially when dealing with deuterium at active positions.

ACKNOWLEDGEMENT

Financial support of this study by Biomedical Support Grant RR07143 is gratefully acknowledged. This is contribution No. 485 from the Barnett Institute of Chemical Analysis.

REFERENCES

- 1 H. Budzikiewicz, C. Djerassi and D. H. Williams, *Structure Elucidation of Natural Products by Mass Spectrometry, Vol. 1: Alkaloids*, Holden-Day, San Francisco, CA, 1964, p. 17.
- 2 M. Senn, W. J. Richter and A. L. Burlingham, *J. Am. Chem. Soc.*, 87 (1965) 680.
- 3 P. Vouros, *J. Org. Chem.*, 38 (1973) 3555.
- 4 G. M. Anthony and G. J. W. Brooks, *J. Chem. Soc.*, D (1970) 200.
- 5 K. Mislow, M. A. W. Glass, H. B. Hopps, E. Simon, and G. H. Wahl, Jr., *J. Am. Chem. Soc.*, 86 (1964) 1710.
- 6 G. J. Kallos and L. B. Westover, *Tetrahedron Lett.*, 13 (1967) 1223.
- 7 W. J. Richter, M. Senn and A. L. Burlingham, *Tetrahedron Lett.* 17 (1965) 1235.
- 8 A. I. Mikaya, V. G. Zaikin, A. V. Antonova and N. S. Prostakoov, *Org. Mass Spectrom.*, 19 (1984) 521.
- 9 D. J. Harvey, M. G. Horning and P. Vouros, *Tetrahedron*, 27 (1971) 4231.
- 10 D. J. Harvey, M. G. Horning and P. Vouros, *J. Chem. Soc., Perkin Trans.*, 1, (1972) 1074.