GAS-LIQUID CHROMATOGRAPHIC DETERMINATION OF RELATIVE AMOUNTS OF 2-DEOXY-2-FLUORO-D-GLUCOSE AND 2-DEOXY-2-FLUORO-D-MANNOSE SYNTHESIZED FROM VARIOUS METHODS

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SUMMARY

A column (6 ft x 1/8 in) containing 4% SE-30 + 6% OV-210 on Chromosorb W-HP 80/100 mesh was employed to determine the relative amounts of 2-deoxy-2-fluoro-D-glucose (2-FDG) and 2-deoxy-2-fluoro-D-mannose (2-FPM) synthesized using various methods. The 2-FDG synthesized from published methods contains 4-56% of 2-FDM.

Key Words: Electrophilic fluorination, 2-Deoxy-2-fluoro-D-glucose, 2-Deoxy-2-fluoro-D-mannose, Gas-liquid chromatographic separations

INTRODUCTION

The 2-^{18}FDG method, first described in 1976 (1-4) has generated widespread interest in the application of this technique for the measurement of regional cerebral glucose metabolism under different pathological states in humans using positron emission tomography (PET). Since that time there have been several other syntheses of this compound employing both electrophilic (5-15) and nucleophilic routes (16-21), and a number of these methods produce radiochemical yields of 2-^{18}FDG which surpass the original method (8-12,14,15,20). Although in each of these methods, isomerically pure 2-^{18}FDG (free of 2-fluoro-2-deoxy-D-mannose (2-FDM), the 2-deoxyglucose derivative where the fluorine atom occupies the axial position) was claimed, four different groups have recently presented experimental evidence that, at least in the case of electrophilic fluorination employing acetyl hypofluorite, varying amounts of 2-FDM itself are produced along with the 2-FDG (22-26). The presence of 2-FDM in the products of these reactions was detected by ^{19}F-NMR (22,24), and by HPLC (23) and GLC (26) examination of

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intermediates in the syntheses. Since ^{19}F-NMR is not readily accessible to many chemists and since it is advantageous to examine the final product(s) of a given reaction rather than intermediates which may vary structurally according to the method used, we have developed a simple gas chromatographic system to separate the readily and rapidly formed trimethylsilyl derivatives of these two fluoro sugars. We report here a description of the separation and its use both to confirm the previous observation and to determine the 2-FDG:2-FDM ratios in other electrophilic fluorination methods.

EXPERIMENTAL

Materials. 3,4,6-Tri-O-acetyl-D-glucal (TAG) and glucal were purchased from Aldrich Chemical Co. and Mara Specialty Chemicals respectively, and were used without further purification. Authentic 2-FDG was obtained from Calbiochem-Behring. 1,1,1,3,3,3-Hexamethyldisilazane and trimethylchlorosilane were purchased from Aldrich Chemical Co.

Chromatography. Gas-liquid chromatographic analyses (GLC) were carried out with a Perkin-Elmer Sigma 300 gas chromatograph equipped with a thermal conductivity detector. A Hewlett-Packard 3390A integrator was used to integrate the peak areas. A column (6 ft x 1/8 in) containing 4% SE-30 + 6% OV-210 on chromosorb W-HP 80/100 mesh from Anspec Co., Inc., Ann Arbor, Michigan was employed, isothermal at 150°C and a flow of 15 ml/min. The 2-FDG and 2-FDM were silylated by the known method (27). The retention times of the silylated derivatives of 2-FDG were 20 min (α) and 28 min (β), while the retention times of 2-FDM derivatives were 23 min (α) and 36 min (β).

RESULTS AND DISCUSSION

There are several common analytical instruments used for the determination of the products purities. These include IR, UV, NMR, MS, HPLC and GLC. Among these, GLC is less expensive, easy to operate and yet has high sensitivity, and is available in almost every laboratory.

We previously had determined the isomeric purity of 2-FDG synthesized by electrophilic fluorinations with the GLC method using a 10% SE-30 column (8,28).

The chromatographic separation of the sample is shown in Figure 1a. On reexamination of the same product mixture, with a 4% SE-30 + 6% OV-210 column (Figure 1b) one can see that the silylated α and β forms of both the 2-FDG and

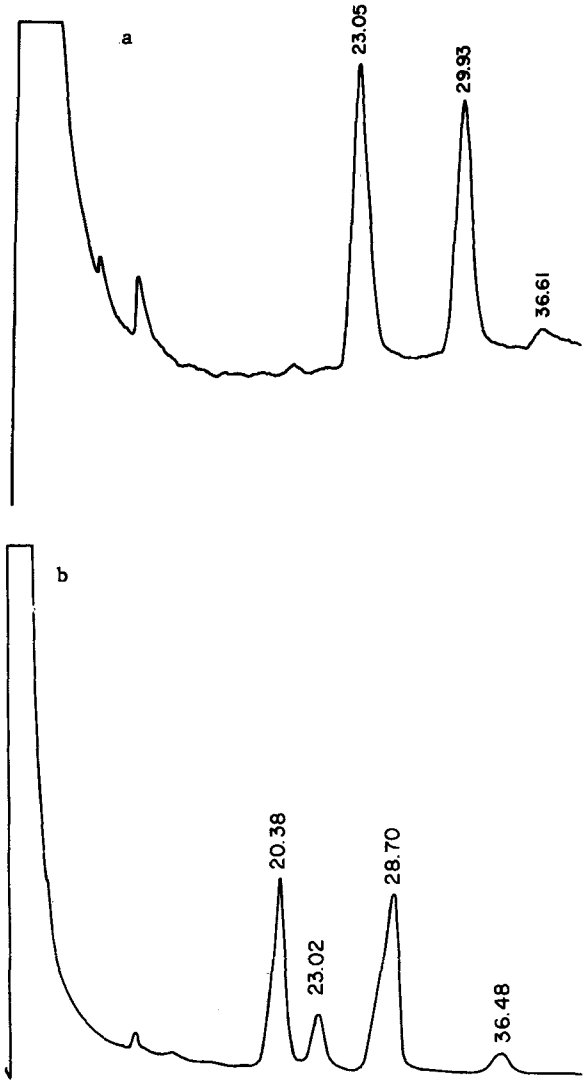

Fig. 1
Mass profile of the gas chromatographic analysis of the product of 3,4,6-tri-O-acetyl-D-glucal with $CH_3CO_2{}^{18}F$ in acetic acid using a 10% SE-30 column (a), and a 4% SE-30 + 6% OV-210 column (b).

the 2-FDM are well separated and that the peak having retention time of 36.6 min in Figure 1a is the β-form of the silylated 2-FDM instead of the third form of silylated 2-FDG as we proposed previously (28).

By using this simple analytical technique, we have determined the relative amounts of 2-FDG and 2-FDM produced using a number of different synthetic methods (Table 1). From all of the 2-FDG preparations we have examined, the reaction of TAG with $CH_3CO_2F(g)$ in Freon-11 produces the least 2-FDM (~ 4%) while the reaction of glucal with $CH_3CO_2F(g)$ produces the most 2-FDM (~ 56%). Perhaps the most surprising result is that even in the original synthesis, where the isomeric gluco and mannopyranosyl fluorides are separated prior to hydrolysis, the product still contains 10% 2-FDM, indicating that the products of this and other electrophilic fluorination mixtures are complex and not easily separated.

Table 1. Relative Amounts (%) of 2-FDG and 2-FDM From Different Sources

Source	References	% 2-FDG	% 2-FDM
TAG $\xrightarrow[CFCl_3,\ -78^oC]{F_2}$	2,3,5,6,7	90	10
TAG $\xrightarrow[CH_3CO_2H]{CH_3CO_2F(\ell)}$	8-10	84	16
TAG $\xrightarrow[ether]{XeF_2}$	13-15	79	21
TAG $\xrightarrow[CFCl_3,\ -78^oC]{CH_3CO_2F(g)}$	22,24 this study	94	6
TAG $\xrightarrow[CFCl_3,\ 25^oC]{CH_3CO_2F(g)}$	This study	96	4
D-Glucal $\xrightarrow[H_2O]{CH_3CO_2F(g)}$	12, 22-25	44	56
Calbiochem-Behring (Lot No. 142003)	This study	92	8
Calbiochem-Behring (Lot No. 410012)	This study	>99	Trace
Samples synthesized by a modification of Tewson's method (20) and supplied by Dr. R. D. Finn	29	>99	Trace

Studies are in progress to determine the steric effects and solvent effects on the stereoselectivity of various electrophilic fluorination reactions with a view to maximizing the 2-FDG:2-FDM ratio, the radiochemical yield, and the chemical and radiochemical purity of 2-^{18}FDG via electrophilic fluorination.

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