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# RECOVERY OF $^{14}$C-LABELED SUGAR AND ALCOHOL DERIVATIVES IN GAS CHROMATOGRAPHY

EUGENE F. JANSEN AND NANCY C. BAGLAN

*Western Utilization Research and Development Division, Agricultural Research Service, U.S. Department of Agriculture, Albany, Calif. (U.S.A.)*

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## SUMMARY

The recovery of trimethylsilyl (TMS) derivatives in gas chromatography was determined by using $^{14}$C-labeled compounds. Under the conditions used approximately one-half of the cholesterol derivative was recovered. Four-fifths of the glycerol derivative and one-fourth of the stearyl alcohol derivative were recovered. Approximately one-fourth of the TMS derivatives of glucose, fructose and sucrose were recovered. Rechromatography of the TMS derivative of glucose resulted in the same loss as found initially. With the TMS derivative of glucose-$^{14}$C, radioactivity was found throughout the length of the column. Thus decomposition and/or deposition of the derivative occurred throughout the column.

The recovery of the trifluoroacetyl derivative of glucose was less than that of the TMS derivative.

## INTRODUCTION

The quantitative analyses of sugars as their trimethylsilyl (TMS) ether derivatives have been based upon the use of internal standards[1,2]. The same is true for the gas chromatography of acetylated alditols derived from sugars[3]. Little effort has been devoted to the determination of the absolute recovery of such derivatives.

SIMMONDS AND LOVELOCK[4] used the ionization cross-section detector as an absolute detector to determine the absolute recovery in the gas chromatography of 32 steroids. They obtained essentially complete recovery only with the hydrocarbon steroid androstane. With cholesterol only 49 to 66% recovery was obtained, depending upon the liquid phase employed. Considerable variations in the percentage recovered of the other steroids examined were observed.

The use of $^{14}$C-labeled sugars and alcohols permitted the determination of the absolute recovery in gas chromatography. The recoveries of the TMS derivatives of $^{14}$C-labeled sugars and alcohols and the trifluoroacetyl (TFA) derivative of glucose were determined and are reported in this paper. It was found that the absolute re-

coveries of these derivatives were considerably less than quantitative and that this was due to decomposition of the derivatives and retention of decomposition products throughout the length of the columns.

## MATERIALS AND METHODS*

Sucrose-$^{14}C$ (u.l., 5 mC per mmole), fructose-$^{14}C$ (u.l., 2 mC per mmole), glucose-$^{14}C$ (u.l., 3 mC per mmole), stearyl-1-$^{14}C$ alcohol (5.3 mC per mmole), cholesterol-4-$^{14}C$ in benzene solution (50 mC per mmole) and glycerol-2-$^{14}C$ in water solution (14 mC per mmole) were purchased from New England Nuclear Corp., Boston, Mass. Silicone Oil SF 96-50, Carbowax 20 M, nonyl-phenoxypolyoxyethylene-ethanol (Igepal) and Chromosorb G were purchased from Varian Aerograph, Walnut Creek, Calif. Sil-Prep ampules containing 1 ml of reagent (pyridine–hexamethyldisilazane–trimethylchlorosilane, 9:3:1) were obtained from Applied Science Laboratories, Inc., State College, Pa.

Radioactivities of $^{14}C$ samples were determined by the liquid scintillation system in naphthalene–dioxane scintillation solution of BUTLER[5] in a Tri-Carb spectrometer manufactured by the Packard Instrument Co., Inc., La Grange, Ill.

A Model 800A gas chromatographic system manufactured by the Packard Instrument Co., Inc. was used. Detection was by the electron capture detector with argon as the carrier gas. All columns were glass coils, 6 ft. by 3 mm I.D. Acid-base washed Chromosorb G 100/120 mesh was the support for the stationary phase. Stationary phases were 2% SF 96-50, 2% SF 96-50 + 0.005% Igepal and 2% Carbowax 20 M with respect to the chromosorb. New columns were conditioned overnight at a temperature slightly above the maximum intended to be used. The argon flow through the columns was approximately 40 cc per min. Injections were made with a Hamilton one-microliter syringe. Collection of the gas effluent was accomplished by means of a short piece of teflon tubing leading directly from the detector outlet into the scintillation solution contained in a vial. Collection was continued for several minutes beyond the end of the detection of the derivative. The teflon tubing was added to the scintillation solution. The radioactivity contained in hexane or tetrahydrofuran solutions of the derivatives was determined by adding aliquots directly to the scintillation solution.

The TMS derivative of cholesterol was prepared with 25 mg of unlabeled cholesterol to which had been added approximately 1 $\mu$C of cholesterol-4-$^{14}C$. The mixture was added to 1.0 ml tetrahydrofuran containing 0.2 ml hexamethyldisilazine and 0.1 ml trimethylchlorosilane. After 20 h at 25° the reaction mixture was evaporated to dryness in vacuum. The reactants were treated with hexane. The solution was filtered, the hexane evaporated and the TMS dissolved in 0.2 ml of tetrahydrofuran and so used in gas chromatography. All other TMS derivatives were prepared with Sil-Prep. One-milliliter ampoules were used with 16.5 mg of unlabeled glycerol to which was added approximately 1 $\mu$C of glycerol-2-$^{14}C$, 15 mg stearyl alcohol containing 1 $\mu$C stearyl-1-$^{14}C$ alcohol, 5 mg sucrose containing approximately 3 $\mu$C of labeled sucrose, 5 mg fructose containing approximately 1 $\mu$C of fructose-$^{14}C$, and 5 mg of

* Reference to a company or product name does not imply approval or recommendation of the product by the U.S. Department of Agriculture to the exclusion of others that may be suitable.

glucose containing from 2 to 20 $\mu$C of glucose-$^{14}$C. Several glucose preparations were used within this range of radioactivity. In every case the TMS derivative after evaporation in vacuum was treated with hexane, filtered, evaporated, and dissolved in approximately 0.2 ml of hexane for gas chromatographic analyses.

The TFA derivative of glucose was prepared by the procedure of TAMURA AND IMANARI[6]. Two preparations were used, one containing approximately 2 and the other 6 $\mu$C of glucose-$^{14}$C per 5 mg of glucose. Hexane solutions of the derivative were used in gas chromatography analyses. In every instance, 1 $\lambda$ of the hexane solutions of TMS and TFA derivatives was injected into the gas chromatography columns.

## RESULTS

The recoveries of the TMS derivatives of $^{14}$C-labeled cholesterol, glycerol and stearyl alcohol are given in Table I. Approximately one-half of the TMS derivative of cholesterol was recovered. This recovery was essentially the same as that obtained with cholesterol by SIMMONDS AND LOVELOCK[4]. Better recoveries (80%) were obtained with the TMS derivative of glycerol. The same recovery was obtained at 105°, 135° and 170°, thus the retention of the glycerol derivative on the column was not temperature dependent. The effect of temperature on the other derivatives was not determined. With the TMS derivative of stearyl alcohol-$^{14}$C only one-fourth of the derivative reached the detector.

TABLE I

RECOVERY OF TRIMETHYLSILYL DERIVATIVES OF CHOLESTEROL-$^{14}$C, GLYCEROL-$^{14}$C AND STEARYL ALCOHOL-$^{14}$C IN GAS CHROMATOGRAPHY

| *TMS derivative* | *Stationary phase* | *Column temperature* | *C.p.m. injected* | *C.p.m. recovered* | *Per cent recovery* |
|---|---|---|---|---|---|
| Cholesterol | SF 96-50 | 250° | 12,300 | 5,300 | 43 |
| | | | 12,300 | 5,500 | 45 |
| | | | 15,200 | 7,200 | 47 |
| | | | 15,200 | 8,200 | 54 |
| | | | 15,200 | 8,500 | 56 |
| Glycerol | Carbowax | 105° | 7,200 | 5,800 | 80 |
| | | | 7,200 | 5,900 | 82 |
| | | | 7,200 | 5,700 | 79 |
| Glycerol | Carbowax | 135° | 6,800 | 4,900 | 72 |
| | | | 6,800 | 5,900 | 87 |
| | | | 6,800 | 5,700 | 84 |
| Glycerol | Carbowax | 170° | 6,800 | 5,400 | 79 |
| Stearyl alcohol | SF 96-50 | 200° | 9,400 | 2,400 | 26 |
| | | | 9,400 | 2,500 | 27 |
| Stearyl alcohol | Carbowax | 200° | 8,900 | 2,500 | 28 |
| | | | 8,900 | 2,700 | 30 |
| | | | 8,900 | 2,100 | 24 |
| | | | 8,900 | 2,000 | 23 |
| | | | 8,900 | 2,700 | 30 |

TABLE II

RECOVERY OF TRIMETHYLSILYL DERIVATIVES OF $^{14}C$-LABELED SUGARS IN GAS CHROMATOGRAPHY

| *TMS derivative* | *Stationary phase* | *Column temperature* | *C.p.m. injected* | *C.p.m. recovered* | *Per cent recovery* |
|---|---|---|---|---|---|
| Glucose | SF 96-50 | 225° | 187,000 | 44,500 | 24 |
| | | | 232,000 | 55,200 | 24 |
| | | | 278,000 | 74,500 | 27 |
| Glucose | Carbowax | 200° | 26,300 | 6,600 | 25 |
| Fructose | SF 96-50 | 225° | 7,800 | 2,000 | 26 |
| | | | 7,800 | 2,800 | 36 |
| | | | 7,800 | 2,100 | 27 |
| Fructose | Carbowax | 200° | 7,800 | 1,800 | 23 |
| Sucrose | SF 96-50 | 225° | 30,400 | 9,400 | 31 |
| | | | 30,400 | 9,400 | 31 |
| | | | 34,400 | 7,400 | 22 |
| | | | 36,700 | 10,800 | 29 |
| | | | 36,700 | 10,800 | 29 |
| Sucrose | Carbowax | 200° | 39,400 | 10,400 | 26 |

The recoveries obtained with the TMS derivatives of sugars (Table II) were less than one-third of that injected. However, the results were reproducible, thus validating the use of internal standards for the analyses of mixtures of sugars as their TMS derivatives.

The recovery of the TMS derivative of glucose-$^{14}C$ on rechromatography was investigated. For this purpose the condensable effluent from the chromatograph of TMS-glucose-$^{14}C$ was collected in a capillary tube. The condensate was combined from several injections. The condensate was dissolved in hexane and rechromatographed. The retention time of the recovered derivative was identical to that observed with the primary hexane solution of the derivative. The recoveries on rechromatography are given in Table III. They were the same as those observed on the first chromatography (Table II). Hence, the material issuing forth from the column was the same as that entering and decomposition apparently occurred in the second chromatography to the same extent as the first.

The site of retention of $^{14}C$ on the column was determined. For this purpose a

TABLE III

RECOVERY OF THE TRIMETHYLSILYL DERIVATIVE OF GLUCOSE-$^{14}C$ ON RECHROMATOGRAPHY

Column: SF 96-50 + Igepal; temperature: 225°.

| *C.p.m. injected* | *C.p.m. recovered* | *Per cent recovery* |
|---|---|---|
| 105,000 | 28,200 | 27 |
| | 27,000 | 26 |
| | 33,500 | 32 |

TABLE IV

RETENTION OF RADIOACTIVITY OF THE TRIMETHYLSILYL DERIVATIVE OF GLUCOSE-$^{14}$C ON THE COLUMN

The stationary phase was Carbowax and the column temperature 200°.

| *Segment No.* | *Amount (cm³)* | *C.p.m.*[a] |
|---|---|---|
| 1[b] | 1 | 1900 |
| 2 | 2 | 1600 |
| 3 | 2 | 1100 |
| 4 | 2 | 690 |
| 5 | 2 | 520 |
| 6 | 2 | 480 |
| 7 | 2 | 280 |
| 8 | 2 | 240 |
| 9 | 2 | 200 |
| 10 | 2 | 170 |
| 11[c] | 2 | 130 |

[a] Corrected for radioactivity contributed by the corresponding amount of stationary phase.
[b] Injection end.
[c] Exit end.

newly prepared and conditioned column was used. The TMS derivative of glucose-$^{14}$C in hexane solution was injected several successive times. The total amount of $^{14}$C injected corresponded to 720,000 c.p.m. The column was cooled and the packing removed in segments beginning at the injection end. The radioactivity in the various segments was determined (Table IV) in thixotropic gel containing scintillation solution*. The $^{14}$C content was greatest at the injection end but nevertheless was found throughout the entire length of the column. Thus decomposition and deposition of the TMS derivative of glucose occurred throughout the column. The total amount of radioactivity found on the column corresponded to only slightly more than 1% of that injected. This figure was minimal because of two considerations. Firstly, the stationary phase *per se* caused considerable quenching when measured by either an

TABLE V

RECOVERY OF THE TRIFLUOROACETYL DERIVATIVE OF GLUCOSE-$^{14}$C IN GAS CHROMATOGRAPHY

| *Column* | *Temperature (°C)* | *C.p.m. injected* | *C.p.m. recovered* | *Per cent recovered* |
|---|---|---|---|---|
| Carbowax | 150 | 70,000 | 9,800 | 14 |
| Carbowax | 170 | 70,000 | 3,600 | 5 |
| | | 23,700 | 2,300 | 10 |
| | | 23,700 | 2,500 | 11 |
| SF 96-50 | 150 | 23,700 | 810 | 3 |
| | | 23,700 | 670 | 3 |
| SF 96-50 | 190 | 23,700 | 1,300 | 5 |

* The radioactivity of 2 cm³ of either the support or the stationary phase in thixotropic gel containing scintillation solution was found to be 100 c.p.m.

internal or external standard. Secondly, the state of deposition of the decomposition products on the stationary phase and the effect thereof on counting efficiency are not known. Answers to these considerations can only be obtained by further and rather extensive research. Of significance was the fact that the $^{14}C$ was distributed all along the column and that decomposition was occurring throughout the column.

A comparison was made between the recovery of TFA and TMS derivatives of glucose-$^{14}C$. The results showed the TFA derivative (Table V) to be inferior to the TMS derivative (Table II). More of the TFA derivative was decomposed and/or retained by the column.

## DISCUSSION

The recoveries of the TMS derivatives of sugars and alcohols were found to be reproducible and sufficiently large to warrant their use in the quantitative determination of such compounds by gas chromatography wherein internal standards are employed. However, because of the low recoveries and the fact that recovery varies from one sugar or alcohol derivative to another, this technique may not be particularly suitable for the separation and identification of an unknown mixture of sugars or alcohols. For example, the method was not satisfactory for the separation of radioactive components contained in the sugar fraction resulting from the metabolism of ethylene-$^{14}C$ by avocado[7] because radioactivity material balances could not be made.

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