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CHEMICAL DERIVATIZATION IN GAS CHROMATOGRAPHY

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CONTENTS

1. INTRODUCTION

Gas chromatography (GC) is suitable for the separation and analysis of substances that display an adequate volatility in the chromatographic system used. The criteria for this general definition are rather loose, as there are a number of ways in which the volatility of a given solute component can be controlled. These aspects have been studied since the advent of GC and, in many respects, it was these problems that led to new concepts in GC techniques and instrumentation and to the development of new chromatographic materials. Examples are high-temperature GC, temperature programming, the use of sorbents of high selectivity, operation in systems with a low content of the sorbent, high-pressure and supercritical-fluid chromatography (up to the transition to liquid chromatography) and chemical conversion of the substances to be chromatographed into more volatile derivatives. The last aspect differs in principle from the others: whereas with the other procedures the volatility of the solute components and its amenability to GC is controlled by changing the properties and operating conditions of the chromatographic system, chemical derivatization alters the properties of the substance to be chromatographed.

When considering the problem of chemical derivatization in GC, it is expedient to distinguish between two causes that can affect the volatility of a substance. Low volatility can be due either to the fact that the substance has large molecules or that the molecules are mutually associated through polar groups. In the first instance, the intermolecular cohesion is the result of interactions by dispersion forces, and the volatility of such compounds obviously cannot be increased by derivatization. In the second instance, however, compounds with relatively small molecules can have very

low volatility if the molecule has functional groups that provide for polar interactions, especially interactions through hydrogen or ionic bonds. Many compounds of this type display a measurable volatility only at temperatures at which they decompose. Some of these compounds are considerably reactive and often decompose on contact with the active surface of a chromatographic support or with metals. These substances almost always give asymmetric chromatographic peaks. In such instances, marked enhancement of volatility and supression of the above undesirable effects can be achieved by effecting a derivatization that blocks the possibility of intermolecular association and reduces the reactivity of the compound. In addition, a conversion into a deliberately chosen derivative may impart to the molecule some properties that provide for selective separation or selective detection.

The combination of GC and chemical derivatization of the substances to be chromatographed is particularly useful in biochemical and biomedical investigations. Because of the wide applicability of GC and the various possibilities of coupling it with other analytical methods, particularly mass spectrometry, it is still of considerable importance although modern liquid chromatography is now used extensively.

This paper reviews the most important work on the preparation and applications of chemical derivatives in analytical GC. In the first part (Section 2), general aspects of the preparation and use of derivatives are dealt with and the most important and most frequently employed derivatives are discussed. In the second part (Section 3), the application of derivatives of individual groups of compounds are considered. The review does not cover reaction GC or pyrolysis GC.

2. GENERAL PART

2.1. Reasons for using chemical derivatives in gas chromatographic analysis

Derivatization is usually carried out in order to increase the volatility of substances with boiling points that are too high, to reduce the adsorption of solutes on the support and column surface and to improve the separation. Special derivatives often provide for the selective detection of certain species of compounds or the separation of chemically very similar compounds, such as optical isomers.

Substances with high molecular weights and several functional groups in the molecule are usually not amenable to GC. Polar functional groups reduce the volatility of the compounds, which results in excessively long retention times or non-elution of the compounds. The volatility can be enhanced by decreasing the polarity by blocking the polar groups, so that the derivatives can be chromatographed with reasonable retention times.

In practice, the reverse case may also occur. It is often necessary to analyze substances of relatively high volatility such as lower carboxylic acids in natural samples, and substantial losses of the components being determined could occur during the preliminary treatment of the sample (extraction, removal of the extractant, etc.). Therefore, the conversion of these compounds into less volatile derivatives is advantageous from the point of view of both the preliminary isolation and the GC determination proper.

Many substances cannot be analyzed by GC owing to their thermal instability. Such substances decompose in the sample inlet port and produce several peaks in the

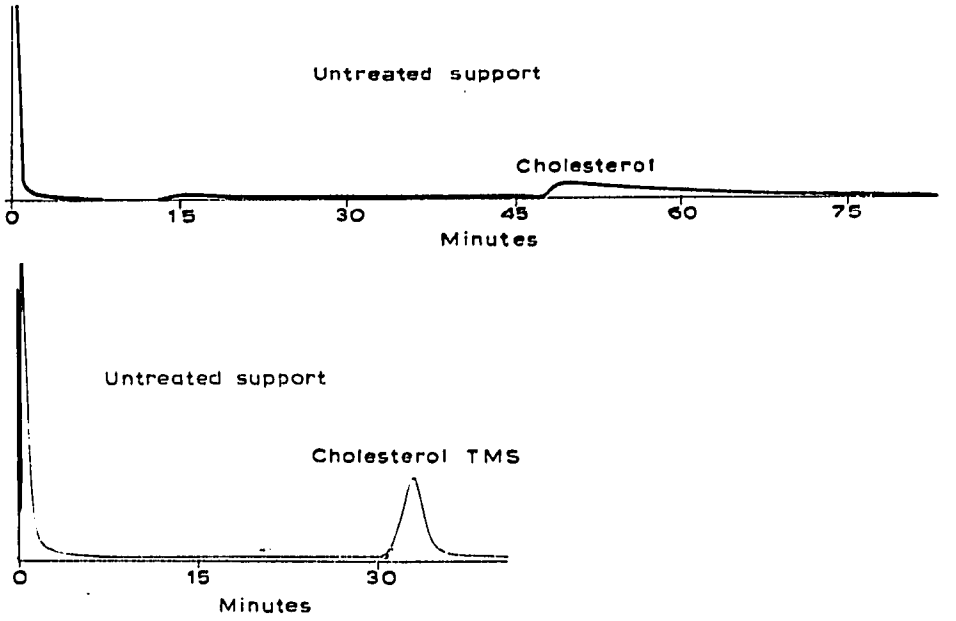


Fig. 1. Comparison of the chromatograms of free and trimethylsilylated cholesterol[557].

chromatogram. These difficulties can also be overcome by using suitable derivatives.

Compounds of high polarity and low volatility are usually very prone to adsorption on the chromatographic support or to decomposition when they come into contact with the latter. In these instances, the quantitative evaluation of the chromatogram is very difficult or even impossible. A classical example is the GC of cholesterol, which can be chromatographed as such or as the trimethylsilyl (TMS) derivative (Fig. 1). The broad and markedly tailing peak of free cholesterol could hardly be evaluated quantitatively, whereas the TMS ether gives a sharp symmetrical peak at an appreciably shorter retention time[557].

Tailing of peaks can also result when the concentration of the solute in the chromatographic system is too high. At high solute concentrations, the sorption isotherm is not linear and the peak is skewed. Even in these instances, the effect can be supressed by using suitable derivatives.

The adsorption of the solute on the support surface or column wall usually results in non-linearity of the calibration graph, especially when working at low solute concentrations in the sorbent and employing peak heights as quantitation parameters. Fig. 2 shows the dependence of the ratios of the peak height of morphine to that of squalene and the peak height of TMS-morphine to that of squalene on the size of the sample charge. With TMS-morphine this dependence is linear, but with free morphine it is non-linear owing to the above adsorption effects. Thus, the use of a suitable derivative can result in a linear dependences of the peak height on the sample charge even at very low solute concentrations[238].

Adsorption on the support and the tailing effect are often caused by carboxyl and hydroxy groups, especially with polyfunctional compounds of higher molecular weight. Amino and imino groups also interact strongly with the support. It is therefore

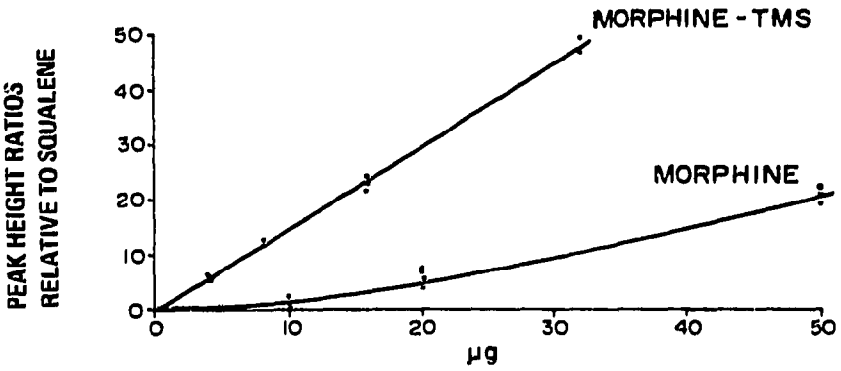


Fig. 2. Effect of silylation on the linearity of the plot of peak height *versus* amount of solute[3,38].

desirable to convert these groups into groups of lower polarity before the analysis proper. The carbonyl group usually does not interact strongly with the support and only rarely presents difficulties. It is derivatized either for special purposes or when its enol form is sufficiently stabilized with the neighbouring groups. Steric hindrance of the polar groups usually reduces their adverse effects, which sometimes may be completely absent so that derivatization is unnecessary. When employing the usual methods of derivatization, the above groups usually react with difficulty, and it is then necessary to employ special procedures.

Derivatization is of great importance in improving the separation of closely related compounds, and it frequently makes it possible to resolve compounds that cannot otherwise be separated. Sterols which differ in the position of the hydroxy group can serve an example. Isomers with a hydroxy group in the α-position are not separated from the β-isomer on non-polar columns, but when the hydroxyl group is

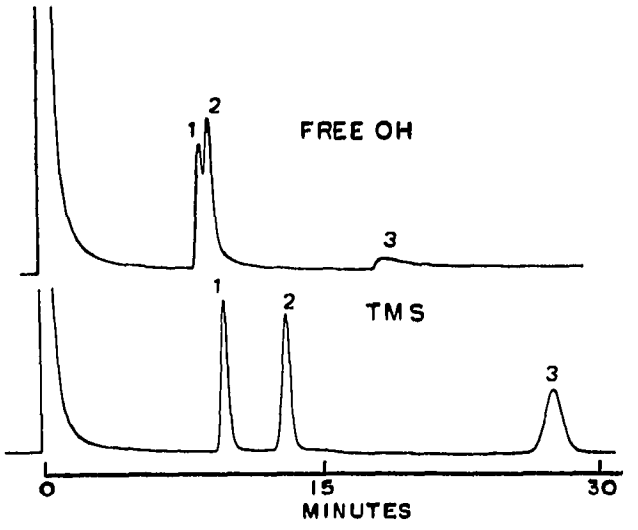


Fig. 3. Effect of silylation on the resolution and peak shapes of estrone (1), estradiol (2) and estriol (3) chromatographed on a non-polar stationary phase[3,57].

converted into a suitable derivative, both isomers are well resolved even on non-polar columns. The separation on a non-polar column of the three estrogens estrone, estradiol and estriol, as such and as their TMS derivatives, is shown in Fig. 3. While only incomplete separation with tailing peaks was achieved with the free compounds, the TMS derivatives were well resolved with symmetrical peaks[557].

Similarly, diverse derivatives can be employed in order to resolve different closely related compounds, such as positional or optical isomers, the separation of which usually presents difficulties. The types of derivative employed and examples are described with the individual groups of compounds in Section 3.

Special derivatives are used in order to increase the detector response. Chlorinated and fluorinated derivatives usually yield very high responses when detected with an electron-capture detector (ECD)[364] and these derivatives are often used in trace analysis. Compounds that contain phosphorus or nitrogen can be used with detectors sensitive to these elements[224].

2.2. General principles of the preparation of derivatives[238]

The preparation of derivatives before GC analysis is a potential source of errors that may affect the entire analytical procedure. For this reason, a thorough knowledge of the reactions used and the factors that influence their results is necessary. Special attention must be paid to the purity of solvents and reagents, the stability of the derivatives and reaction rates.

2.2.1. Technique of sampling

The method of sampling may be a serious source of errors in any analytical method and is critical in GC analysis. In addition to the problems of ensuring adequate homogeneity of the sample and taking a representative sample, there is a problem of its transfer into the gas chromatograph. It is necessary to ensure that the sample is not subject to decomposition or any reaction prior to the preparation of derivatives or the injection of a sample charge into the gas chromatograph. Items that are decisive for the success of the method are a knowledge of the kinetics of the reactions involved, removal of impurities from the solvents and reagents employed and, if necessary, the use of stabilizers. Although these aspects may seem obvious, it is because they are not strictly adhered to that many workers do not obtain satisfactory results.

2.2.2. Purity of solvents and reagents

Impurities that are introduced into the sample during cleaning, extraction, etc., appear as interfering peaks in the chromatogram and complicate the analysis or even make it impossible. If thin-layer chromatography (TLC) is employed for the preliminary clean-up of the sample, a blank experiment should be carried out by determining the impurities present in the adsorbent used; after the extraction of an amount of the adsorbent with the solvent, the condensed extract is derivatized and analyzed in the same manner as in the GC analysis proper. Interfering substances in the solvents and other chemicals used in the analysis are determined in a similar way. If some of the materials employed give peaks that overlap with those derived from the sample being analyzed, it is necessary to use chemicals of a higher grade of purity or, if necessary, to re-purify them.

2.2.3. Stability of derivatives

Some derivatives decompose by the action of heat, moisture, light, etc., when stored for a long period. In these instances, derivatization has to be carried out just before the analysis. This prerequisite is particularly important with derivatives that are very sensitive to moisture, such as trimethylsilyl derivatives.

2.2.4. Reaction rate

If quantitative analysis is concerned, the degree of conversion in the reactions involved should be known. Usually it is not known, and it is then necessary to assume that the reaction rates with the standard and the component being determined are identical. In this event, however, the conditions of reaction, such as the temperature, reaction time, type of catalyst and concentrations of the reactants, must also be the same in each instance. Sometimes the products of the decomposition of reagents may adversely affect the yield of a reaction and it is then necessary to store the chemicals in such a way that they can always be made available in a sufficiently fresh state.

2.3. Derivatives employed

2.3.1. Esters

Methyl esters are the most commonly used derivatives of the carboxylic group. The volatility of methyl esters is sufficiently high to permit the GC determination of even higher fatty acids[572]. However, with short-chain fatty acids, the volatility of the methyl esters is unsatisfactory as it can cause losses of the derivatives before analysis. Methyl esters are usually sufficiently stable, only the esters of some keto acids decomposing at about 100° in GC[172].

There are several methods for the preparation of methyl esters. Perhaps the most widely used are those which employ diazomethane[456,468] and a methanolic solution of boron trifluoride[394,410]. Also frequently used are reactions with hydrochloric acid and methanol[526] and sulphuric acid and methanol[359], by which means triglycerides are converted directly into the methyl esters of their fatty acids. Further, methyl esters are prepared by the pyrolysis of tetramethylammonium salts in the injection port[31,256], by methylation with 2,2-dimethoxypropane with the addition of dimethyl sulphoxide in order to inhibit the polymerization of the reagent[509], methylation on an ion exchanger[282] and other methods[106,157,585].

Vorbeck *et al.*[572] compared the yields obtained with different methylation methods (Table 1). With both lower and higher fatty acids, the best results were obtained by using the diazomethane method.

Diazomethane method. The reaction proceeds as follows:

$$R\text{–}COOH + CH_2{=}N{=}N \rightarrow R\text{–}COOCH_3 + N_2$$

Usually use is made of an ethereal solution of diazomethane prepared by the decomposition of N-nitroso-N-methyl-*p*-toluenesulphonamide with alkali. The ethereal solution of diazomethane is added gradually to the ethereal solution of the compounds to be derivatized until the reaction mixture remains permanently yellow. Another method consists in using three mutually connected bubble-through vessels which are swept with dry nitrogen. The first vessel contains diethyl ether, the second an

TABLE 1

COMPARISON OF YIELDS OBTAINED IN DIFFERENT METHODS OF METHYLATION

For butyric to caproic and for myristic to linoeic acids, the standard deviations were 0.32, 26.5 and 13.8, and 0.25, 0.77 and 0.52% with diazomethane, CH_3OH–HCl and CH_3OH–BF_3, respectively.

Acid	*Concentration given (wt.-%)*	*Concentration found (wt.-%)*		
		Diazomethane	*CH_3OH–HCl*	*CH_3OH–BF_3*
Butyric	38.7	38.6	4.0	30.1
Valeric	30.0	29.8	14.8	24.4
Caproic	31.1	31.6	29.6	30.4
Myristic	14.2	14.7	13.8	13.9
Palmitic	16.1	15.9	15.7	15.6
Stearic	17.8	17.6	18.7	18.0
Oleic	17.8	17.6	18.7	18.0
Linoleic	17.8	17.8	17.8	17.8

ethereal solution of 2-(2-ethoxyethoxy)ethanol plus an aqueous solution of potassium hydroxide, and the third an ethanolic solution of the acid. On adding N-nitroso-N-methyl-*p*-toluenesulphonamide to the second vessel, diazomethane is evolved and introduced into the third solution until a yellow coloration is attained. The excess of diazomethane is removed with a stream of pure nitrogen[487]. Fales *et al.*[175] used successfully the apparatus shown in Fig. 4. After injecting the reagents through the septum, the diazomethane produced within the inner tube dissolves in the diethyl ether or other liquor at the bottom of the other tube. The lower part of the apparatus is chilled with ice.

The diazomethane method is advantageous because of its simplicity and the

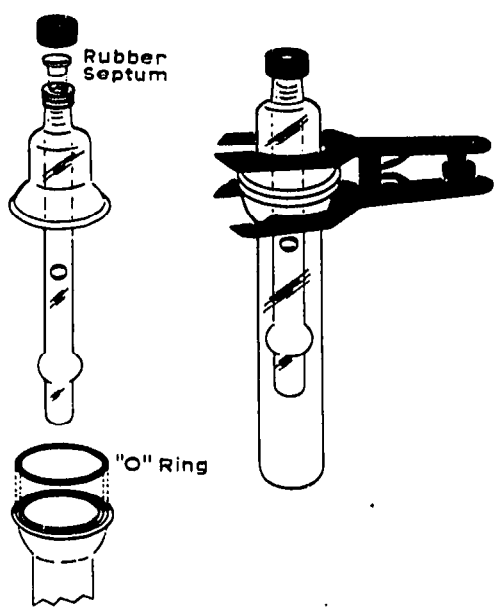


Fig. 4. Arrangement for methylation with diazomethane[175].

ease of preparation of methyl esters. As diazomethane reacts with water, it is necessary to work in water-free media. The formation of polymethylene polymers[572] can be prevented by employing vessels with a clean and smooth inner surface. A disadvantage of diazomethane is its toxicity and high reactivity, which often causes explosions. Therefore, ethereal solutions of diazomethane must be either used immediately or stored for only a short period at −20°.

Methanol method: catalysis with boron trifluoride. This method is represented by:

$$R\text{-}COOH + CH_3OH \xrightarrow{BF_3} R\text{-}COOCH_3 + H_2O$$

After adding the reagent to the sample of the acid, the reaction is completed by boiling the mixture for 2 min on a water-bath. On adding diethyl ether and water, the mixture distributes itself into two phases. After the removal of the aqueous layer, the ethereal phase is filtered, concentrated by heating on a water-bath and chromatographed. The reagents used are readily available and the mixture is sufficiently reactive even towards strongly hindered groups[394]. In some instances, boron trichloride[67] is more suitable than boron trifluoride.

Methanol method: catalysis with hydrochloric or sulphuric acid. The reaction is

$$R\text{-}COOH + CH_3OH \xrightarrow{HCl/H_2SO_4} R\text{-}COOCH_3 + H_2O$$

The preparation is carried out as in the preceding case. The methanolic solution of the sample is mixed with hydrochloric or sulphuric acid and is then refluxed for 2 h on a water-bath. The reaction mixture is again extracted with diethyl ether and the extract is cleaned, condensed and eventually injected into the gas chromatograph.

Pyrolysis of tetramethylammonium salts. This method is based on the reaction

$$R\text{-}COOH + (CH_3)_4N\text{-}OH \xrightarrow{360\text{–}400^\circ} R\text{-}COOCH_3 + H_2O + (CH_3)_3N$$

Usually, a sample of the acid is titrated with a methanolic solution of tetramethylammonium hydroxide on phenolphthalein. The solution is either injected directly into the GC inlet port warmed up to 360–400°[31], or placed in a 3-μl capillary, dried at 100° and eventually pyrolyzed at a higher temperature. Trimethylanilinium hydroxide in methanol can also be used as the reagent[69,536].

Higher esters are used for the GC of lower acids, where a higher volatility of the methyl esters can cause losses during preparation and erroneous quantitative results. Butyl[215] and benzyl[332] esters are very frequently used, but other esters have also been studied: ethyl and propyl esters[22], substituted benzyl esters[556,578], esters that give a high response with the electron-capture detector, such as β-chloroethyl[312], pentafluorobenzyl[319], and hexafluoroisopropyl esters[160], and others. L-Menthyl esters[1] were used to distinguish optical antipodes.

The methods of the preparation of higher esters are similar to those for methyl esters. A commonly used method is the esterification of the carboxylic group by reaction with an alcohol, catalyzed by boron trifluoride or hydrochloric or sul-

phuric acid. Felder *et al.*[178] esterified a number of acids with methanol and higher alcohols. The reaction was catalyzed by pyridine with a large excess of N,N'-dicyclohexylcarbodiimide, which acted as a dehydration agent:

$$RN{=}C{=}NR \xrightarrow{H^+} RN{=}C{=}\overset{+}{N}HR \xrightarrow{R_1COO^-} RN{=}C(O{-}CO{-}R_1){-}NHR \xrightarrow{R_2OH} R_1{-}COOR_2 + RNH{-}CO{-}NHR$$

If a precipitate of dicyclohexylurea is produced in the reaction, it is allowed to settle and the clear solution is injected into the gas chromatograph. Felder *et al.*[178] obtained quantitative results with a number of fatty, pyrazine and halobenzoic acids. The use of higher diazoalkanes[118,587] or diazotoluene[117] has also been described. Their ethereal solutions are not as explosive as those of diazomethane and they can be stored at low temperatures for longer periods. Good results were achieved by Thenot *et al.*[548], who esterified carboxylic acids by reaction with N,N'-dimethylformamide acetals:

$$R{-}COOH + CH(O{-}R')_2{-}N(CH_3)_2 \xrightarrow{-R'{-}OH} R{-}COO^- + R'{-}O{-}CH{-}\overset{+}{N}(CH_3)_2 \rightarrow RCOOR' + HCON(CH_3)_2$$

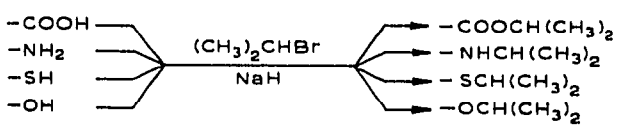
The reaction is sufficiently quantitative for analytical purposes and the alkyl group R' can be varied widely.

Other methods of preparation of esters for GC include the alcoholysis of the imidazolides of acids, which are prepared by the reaction of the acid with N,N'-carbonyldiimidazole[335]:

and the reaction of alkyl iodides with the tetramethylammonium salts of acids[223]:

$$R{-}COOH \xrightarrow{(CH_3)_4N^+OH^-} R{-}COO^-(CH_3)_4N^+ \xrightarrow{R'{-}I} R{-}COOR'$$

Special mention can be made of isopropyl esters, which are prepared by reaction with 2-bromopropane and sodium hydride. These derivatives were introduced by Pettitt and Stouffer[448]. It is interesting that this mixture of reagents can also derivatize other functional groups in the molecule, which can be of practical importance in the analysis of compounds with different functional groups:



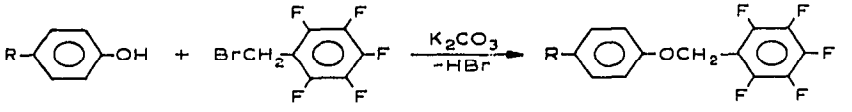
Up to now this reaction has been little utilized for derivatization in GC analysis, and it may possibly find wider application.

2.3.2. Ethers

This type of derivatization is used to protect hydroxy groups. Apart from TMS ethers, which will be dealt with separately, ethers have not been widely used as derivatives in GC analysis and are employed for special purposes. Methyl ethers of saccharides are prepared by reaction with methyl iodide in the presence of silver(I) oxide in dimethylformamide[325]. An ethereal solution of potassium *tert.*-butanolate has been used[110] instead of silver(I) oxide.

$$2\ R\text{–}OH + 2\ CH_3I + Ag_2O \xrightarrow{\text{catalyst}} 2\ R\text{–}OCH_3 + 2\ AgI + H_2O$$

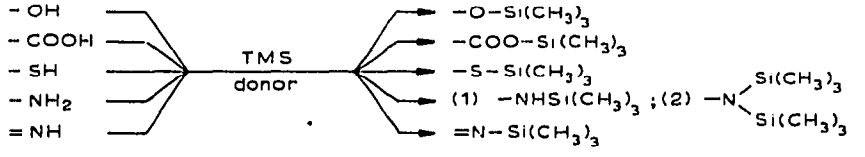
Earlier, the method was often used mainly to derivatize high-molecular-weight hydroxy and polyhydroxy compounds. Derivatives for the trace analysis of hydroxy compounds are prepared in a similar way; the reaction of phenolic compounds with α-bromo-2,3,4,5,6-pentafluorotoluene results in an ether that gives a high ECD response. This reaction is catalyzed by potassium carbonate[318]:



Similarly, reaction with 1-fluoro-2,4-dinitrobenzene gives 2,4-dinitrophenyl ethers[112].

2.3.3. Silyl derivatives

These derivatives have been most widely used in the GC of non-volatile substances. In particular, the trimethylsilyl (TMS) group can be used in order to block diverse polar groups. The preparation of the derivatives is represented by the scheme



If the enolized carbonyl group is included with the above range of functional groups, virtually all groups that may complicate the GC analysis owing to their polarity are covered. The advantage of trimethylsilylation is evident particularly with compounds that have different functional groups in the molecule, as all of the groups can be derivatized by a one-step reaction.

The methods of preparation of TMS derivatives have been described in detail. In his monograph, Pierce[449] presented a number of different modifications of the preparations of TMS derivatives according to the reagents and functional groups. The following reagents were used:

(1) trimethylchlorosilane (TMCS), alone or with an acceptor of the acid or, if necessary, with a catalyst;

(2) hexamethyldisilazane (HMDS), mostly with TMCS or another catalyst;

(3) silylamines, such as trimethylsilyldiethylamine (TMSDEA) and trimethylsilylimidazole (TMSIM);

(4) silylamides and other reagents: most commonly employed are N,O-bistrimethylsilylacetamide (BSA), N,O-bistrimethylsilyltrifluoroacetamide (BSTFA) and hexamethyldisiloxane (HMDSO).

The frequently used mixture of HMDS and TMCS in pyridine[532] is a relatively weak TMS donor; stronger reagents, mainly BSA, BSTFA[523] and TMSIM, are employed in order to derivatize strongly hindered groups and groups of low reactivity. Other analogous reagents, N-methyl-N-TMS-trifluoroacetamide and N,N,N',N'-tetrakis-TMS-1,*n*-diaminoalkanes, were introduced by Donike[144,145].

Pyridine is often used as reaction medium, but it is difficult to chromatograph owing to the tailing of peaks and its peak can overlap with some lower derivatives. Other solvents used include acetonitrile and dimethylformamide. During derivatization, it is necessary to maintain strictly anhydrous conditions as even trace amounts of water decompose TMS derivatives into the parent compounds. Weiss and Tambawala[580] described a method of silylation in the presence of water, but the principle consists in adding such a large excess of the reagent that the amount of water present becomes negligible.

The reaction is usually performed in flasks sealed with silicone rubber closures, and the introduction of the reagent and withdrawal of samples are carried out through the stepum with the use of an injection syringe. Because of the sensitivity of the derivatives towards water, it is necessary to prepare them immediately before analysis, although the derivatives have been reported to remain stable for several days under anhydrous conditions[276]. A method has also been described for the preparation of TMS derivatives on the column[168,387]. The sample charge is followed by a charge of the silylation agent, the conditions being chosen such that the components to be separated are freed from water and alcohol when entering into the reaction. The TMS derivatives produced in this way then migrate down the column and are separated. An apparatus for the removal of pyridine from the sample was described by Lehrfeld[355].

The choice of the stationary phase for the separation of TMS derivatives is usually not critical. Use has been made of diverse stationary phases, but non-polar and non-selective phases are to be preferred[449]. The support is usually deactivated, by washing it with an acid followed by silanization, which helps to produce a higher separation efficiency of the column[397]. The packing must not be acidic in case the derivatives decompose. Some less stable derivatives can decompose upon contact with metallic parts of the instrument and it is therefore recommended that all-glass apparatus be employed; the column, however, may be made of glass or stainless steel[205]. When using a flame-ionization detector, an aerosol of silicon dioxide is produced on combustion of siliceous substances and is deposited on the electrodes; this deposit can decrease the sensitivity of detection or alter the response factors. When using BSTFA, this effect is reduced owing to the formation of volatile silicon tetrafluoride.

Halomethyldimethylsilyl derivatives and dimethylsilyl derivatives[530,531] are prepared in a similar way. They have shorter retention times than TMS derivatives, but are less stable[238]. Some examples are shown in Table 2.

TABLE 2

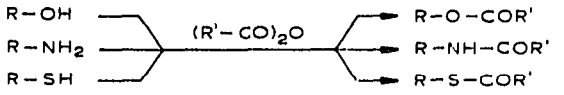
COMPARISON OF RETENTION TIMES OF SOME HIGHER ALCOHOLS, PHENOLS AND THEIR DMS AND TMS DERIVATIVES

Conditions: 6 ft. × 4 mm I.D. glass column; 15% Apiezon L on Gas-Chrom P, 100–120 mesh; 120°; nitrogen carrier gas, flow-rate 60 ml/min. Retention times are given relative to hexadecanol and its derivatives and phenol derivatives, respectively. Values in parentheses are absolute retention times.

Compound studied	*Relative retention time*		
	Parent compound	*DMS derivative*	*TMS derivative*
Dodecanol	0.17	0.19	0.19
Tetradecanol	0.42	0.43	0.44
Hexadecanol	1.00	1.00	1.00
	(14.0 min)	(8.9 min)	(10.4 min)
Octadecanol	—	2.29	2.28
Phenol	—	1.00	1.00
	—	(8.5 min)	(11.2 min)
o-Cresol	—	1.73	1.74
m-Cresol	—	1.85	1.78
p-Cresol	—	1.98	1.98

2.3.4. Acyl derivatives

Acyl derivatives are common derivatives of hydroxy, amino and thiol groups:



Halogenated acyl derivatives have found the widest application owing to their high ECD response and utility in trace analysis[447].

Acyl derivatives are prepared by reaction with an excess of the acylation reagent (usually the anhydride of the corresponding acid) in pyridine, tetrahydrofuran or another solvent that is able to bind the acid produced. The type and amount of solvent used frequently have a significant influence on the yield of the reaction[459]. The reaction mixture is then usually heated for 1/2–1 h at 60° in order to evaporate the solvent, and the concentrate is injected into the gas chromatograph. It is essential to work under anhydrous conditions as the derivatives are hydrolyzed on contact with water. Trace amounts of water are removed by the excess of solvent, which protects the derivatives against hydrolysis. The chloride of an acid can also be used as an acylation agent. Advantageous acylation agents are acylimidazoles[279]; by-product imidazole is relatively inert and does not decompose the derivatives. Anders and Mannering[15] prepared acetyl and propionyl derivatives directly in the column by injecting the sample and anhydride consecutively. Different retention times of the derivatives were utilized in order to identify some alkaloids and steroids.

Acetyl derivatives. Acetyl derivatives were mainly used in earlier work. Their significance lies in the ready availability of the reagents. Nowadays, halogenated acetyl derivatives are more common owing to their high affinity for electrons and the possibility of carrying out high-sensitivity analyses with the use of ECDs. Landowne and Lipsky[346] arranged the haloacetyl esters of sterols in order of increasing ECD response: trifluoroacetate < trichloroacetate < bromoacetate < dichloroacetate <

chloroacetate. However, chloroacetyl derivatives have some unfavourable properties, such as the formation of asymmetric peaks[108], so that trifluoroacetyl (TFA) derivatives are used more frequently. In addition to the above methods, TFA derivatives can be prepared by a method described by Donike[146]: trifluoroacetylation is carried out with N-methyl-bis-TFA-amide, which reacts with $-NH_2$, -OH and -SH groups under mild conditions. As the reagent is a liquid, no solvent is necessary. The excess of the reagent protects the derivatives against hydrolysis.

Halogenated acyl derivatives of higher acids usually afford as a rule even more sensitive analyses than do TFA derivatives. McCallum and Armstrong[384] compared the responses of seven derivatives of thymol: 2,4-dinitrophenyl and pentafluorobenzyl ethers and heptafluorobutyryl, pentafluoropropionyl, chloroacetyl, fluoroacetyl and pentafluorobenzoyl esters. The most sensitive analysis was achieved with pentafluorobenzoate, with which it was possible to determine as little as 1 pg of thymol. The above situation is shown in Table 3. Heptafluorobutyrates (HFB) give a lower sensitivity, but they have very high volatility and are used in practice mainly with natural amines and alcohols of low volatility[595].

TABLE 3

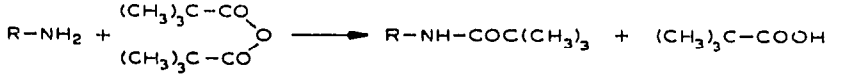
RELATIVE SENSITIVITY OF THE ELECTRON-CAPTURE DETECTOR TOWARDS DIFFERENT DERIVATIVES OF THYMOL[384]

Conditions: 1 m × 2 mm I.D. glass column; 1% SE-52 on Diatoport S; nitrogen carrier gas, flow-rate 15–20 ml/min.

Derivative	*Column temperature (°C)*	*Retention time (min)*	*Relative sensitivity**
Heptafluorobutyrate	70	2.7	1.0
Pentafluoropropionate	70	1.9	1.3
Monochloroacetate	100	3.1	0.3
Monofluoroacetate	100	1.2	$7 \cdot 10^{-3}$
Pentafluorobenzyl ether	100	5.8	5.9
Pentafluorobenzoate	150	1.7	6.9
2,4-Dinitrophenyl ether	150	9.8	0.3
Free thymol detected with FID	120	3.0	$7 \cdot 10^{-4}$**

* Relative to heptafluorobutyrate.
** Relative to the ECD response of thymol heptafluorobutyrate.

N,O-Dipivalyl esters are prepared by a procedure similar to that described above, and have been used for the analysis of thyroid hormones[288]. Both amino and hydroxy groups are converted into the pivalyl derivative:



The derivatives are stable and can be cleaned up prior to GC, *e.g.*, by TLC.

2.3.5. Oximes and hydrazones

The carbonyl group usually does not present any special difficulties in GC

analysis. However, the peaks of carbonyl compounds often overlap with those of interfering components[570], or the presence of the carbonyl group can sometimes be the cause of instability of compounds and bring about asymmetric peaks. In these instances, the carbonyl group must be converted into an inert derivative. Oximes proved to serve this purpose very well:

They are usually prepared by reaction of the reagent (hydroxylamine, methoxylamine or benzyloxyamine hydrochloride) with the carbonyl compound in pyridine. The reaction is allowed to proceed either at ambient temperature overnight or is accelerated by warming the mixture to 60–100°. The pyridine is removed with a stream of nitrogen and the sample is dissolved in another solvent (ethyl acetate) prior to its introduction into the gas chromatograph; if necessary, other groups can be blocked. Oximes alone (R = H) are used only rarely. Lohr and Warren[363] noticed that different oximes decomposed to the corresponding nitriles on the column, the decomposition being dependent on temperature. At 250°, the decomposition is complete and the chromatograms of the products are reproducible. However, methoximes have found much wider application[546], particularly for protecting labile keto groups in substances with higher molecular weights (steroids). Compared with the parent compounds, methoximes are more stable, do not decompose during analysis and can be further modified chemically according to the type of interfering group. Higher oximes, such as O-butyloximes, O-pentyloximes and O-benzyloximes, have also been studied and are important when combining GC with mass spectrometry. They are sufficiently stable and their characteristic mass spectra can be easily interpreted quantitatively[32].

Good chromatographic properties have been encountered with hydrazones of carbonyl compounds:

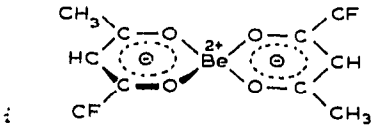
They are prepared by the reaction of a substituted hydrazone with the sample in the presence of a catalyst, usually acetic acid. At ambient temperature, the conversion is complete in 1–2 h. The excess of the reagent is usually removed with a stream of nitrogen and the derivative, dissolved in a suitable solvent, is injected into the gas chromatograph. The derivatives can also be prepared by use of Girard T reagent[199]. The direct GC analysis of hydrazones is carried out only with special derivatives. For instance, 2,4-dinitrophenylhydrazones and 2,4,6-trichlorophenylhydrazones have a sufficiently high ECD response and can be used in trace analysis[297]. In addition, hydrazones are used for the preliminary isolation of carbonyl compounds; in GC analysis, they are injected together with α-ketoglutaric acid or another keto compound that liberates the carbonyl compound, the latter being chromatographed in its free state.

2.3.6. Chelates of metals

Ions of metals can be analyzed by GC in the form of their volatile compounds. Chlorides[528,604] and fluorides[306] have sufficient volatility for this purpose, but special

reactors have to be installed ahead of the column. Jones and Nickless employed arylmercury[301] and methylmercury[302] compounds for the determination of mercury, and Tatton and Wagstaffe[538] analyzed dithizonates of mercury. Segard *et al.*[494] described the GC separation of 13 arenetricarbonic complexes of chromium. However, these compounds are interesting from the point of view of the chemistry of complexes rather than for their analytical utility. Schwedt and Ruessel[491] converted arsenic in biological tissues into triphenylarsine and analyzed it by GC.

Chelates of metals have found much wider application. They are prepared by using β-diketones of the acetylacetone type[507], but other similar compounds, such as β-ketoamines[269] and monothioacetylacetone[38], can also be employed. The active hydrogen of the methylene group of β-diketones is substituted by the metal, and the second co-ordination bond is formed by the oxygen of the ulterior keto group. This gives rise to a stable six-membered heterocycle; an example of a beryllium complex shows the saturation of four co-ordination bonds by two molecules of β-diketone:



Chelates are prepared by reaction of the diketone with the sample, the mixture being extracted (usually with benzene) and the extract chromatographed. By use of fluorinated diketones and an ECD, it is possible to attain a high sensitivity of analysis, but the linearity of the response is poor[5,477].

The earliest used and most studied chelate-producing reagent is acetylacetone and its trifluoro and hexafluoro derivatives. These reagents have been used for the preparation and determination of complexes of beryllium[329,475,507], aluminium[407,493,507], chromium[214,476,478], copper and iron[329,493], rare-earth metals[507] and other metals[407]. Several workers have demonstrated the application of this approach to the determination of toxic beryllium in diverse biological materials[541,592], blood[540] and urine[186]. Eisentraut *et al.*[165] determined beryllium in lunar and meteoric samples by this means. The high-sensitivity determination of chromium in biological samples[62,245,474,485] has also been described.

Higher volatility is displayed by chelates with pivaloyltrifluoroacetylacetone (2,2-dimethyl-6,6,6-trifluorohexanedione-3,5), used for the determination of rare-earth metals[501,537] and other trivalent metals[41]. 2,2,6,6-Tetramethylheptadione-3,5 (THD) has been employed with rare-earth metals[490]. In the analysis of calcium and strontium, the situation is complicated by the formation of mixed chelates (dimers):

$$[Ca(THD)_2]_2 + [Sr(THD)_2]_2 \rightleftharpoons 2\ CaSr(THD)_4$$

Highly fluorinated chelate-producing agents are employed in trace analysis with the use of an ECD. 1,1,1,2,2,3,3-Heptafluoro-7,7-dimethyloctadione-4,6 forms, with rare-earth metals, iron, nickel, chromium, copper and other metals, complexes that have excellent chromatographic properties, high volatility and stability and a high ECD response, enabling the ultramicro analysis of these elements to be performed[185,506,521]. Highly fluorinated diketones, such as decafluoroheptanedione-3,5 and dodecafluorooctanedione-4,6, were employed in the preparation and high-sensitivity analysis of

mixed complexes of rare earths; tri-*n*-butyl phosphate and di-*n*-butyl sulphoxide were used as partner ligands. In addition to lanthanides[34,84,88], uranyl and thorium[505] have been determined in this way.

Of other reagents, use has been made of hexafluoromonothioacetylacetone for the preparation of volatile derivatives of platinum metals[38] and nickel[35,44] in particular, and of bisacetylacetone methylenediimine for nickel, palladium and platinum[43]. Further references and information can be found in a monograph by Moshier and Sievers[412] published in 1965, and in more recent reviews[42,123].

3. SYSTEMATIC PART

3.1. Alcohols and phenols

Although the GC analysis of lower alcohols is no longer a major problem, the presence of the hydroxy group results in considerable adsorption on some kinds of support, which leads to peak tailing and renders quantitative analysis impossible, especially with aromatic hydroxy compounds. In addition, in trace analysis it is necessary to prepare derivatives with a high detector response and, in the analysis of optical antipodes, derivatives that will permit their resolution. Zarazir *et al.*[599] presented the retention data of various derivatives of a number of alcohols on three stationary phases. On plotting these data on a triangular diagram, it was possible to identify unknown compounds.

3.1.1. Esters

Studies have been made of the acetates of alcohols[232], glycols and polyethylene glycols[233] and some phenolic compounds[217] as the most accessible derivatives. Decroix *et al.*[136] used benzoyl esters for the determination of glycerol, as it is not necessary to use an anhydrous medium. However, the derivatives decompose on the column and special conditions must be observed during the analysis.

Halogenated acyl derivatives are frequently employed in order to achieve selective detection. Argauer[23] described the GC of 32 phenols as their chloroacetyl esters, and Larkham and Pagington[351] used these derivatives to prove the presence of trace amounts of alcohols in tobacco smoke. TFA esters of phenols have also been studied, but they are rather unstable[503]. Trace amounts of water and acids cause their decomposition, and sterically hindered groups do not react quantitatively. These derivatives are of advantage with compounds that have different functional groups, for instance in the separation of ethanolamines[81] and metabolites of catecholamines[17]. Walle and Ehrsson[574] used HFB esters for the determination of picogram amounts of various alcoholic compounds.

Interesting derivatives have been described by Bassette *et al.*[37]. In order to increase the sensitivity of the determination of primary and secondary alcohols, they employed 2,6-dinitrophenylhydrazones of pyruvic acid. Neurath and Lüttich[423] studied the GC separation of esters of 4'-nitroazobenzene-4-carboxylic acid and gave the corresponding data for a number of alcoholic compounds.

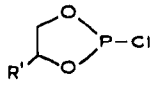
3.1.2. Ethers

Although phenolic hydroxy compounds were separated as the methyl esters in

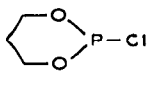
early work[93,94], these derivatives are used only in special cases today (except for TMS ethers), particularly for increasing the sensitivity of the determination. Haken and Khemangkorn[233] studied the GC behaviour of different ethers and ether acetates of propylene and ethylene glycols. Kawahara[318] investigated pentafluorotolyl ethers of several alcohols and phenols, which derivatives he prepared by using α-bromo-2,3,4,5,6-pentafluorotoluene, and showed that they had a number of excellent chromatographic properties. These ethers are stable in water, give an excellent ECD response, which is specific in the presence of impurities, and they are particularly suitable for trace analysis. Other workers have studied 2,4-dinitrophenyl ethers[112] and 2,6-dinitro-4-fluoromethylphenyl ethers[495] of phenols. The quantitativeness of their preparation is worse than that of the above derivatives, the yields varying in the range 50–70%, depending on the type of phenol involved.

3.1.3. Heterocyclic derivatives

Vilceanu and Schulz[567] developed phosphorus-containing heterocyclic derivatives for the high-sensitivity detection of lower alcohols with an alkali flame-ionization detector. The derivatives (III, IV) were prepared by reaction with 2-chloro-1,3,2-dioxaphospholane (I) and 2-chloro-1,3,2-dioxaphosphorinane (II):



(I)



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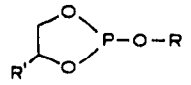
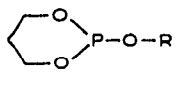


(III)



(IV)

These derivatives are especially suitable for the determination of alcohols in anhydrous and alcohol-free media.

3.1.4. Trimethylsilyl derivatives

These derivatives have been studied in detail in the GC of alcohols, owing to their chromatographic properties and easy preparation. As the reactivity of the hydroxyl group is sufficiently high, except for some special instances, it is generally possible to employ any silylation agent. For the derivatization of the less reactive tertiary alcohols, the formerly used HMDS[348,349] has been replaced by a more reactive mixture of HMDS and TMCS[191]. The preparation of volatile derivatives of glycols[356] and polyethylene glycols[24,169,591] is carried out mainly with BSA or, if necessary, BSTFA. The use of these derivatives in the determination of glycerol in lipids[458], higher terpenic alcohols[496,579] and fatty alcohols[593,601] has been studied. With some compounds, *e.g.*, prostaglandins, use was made, in addition to TMS ethers alone[305], of a combination of them with methyl or acetyl derivatives[6] or with cyclic *n*-butyl boronates[433]. The TMS derivatives of phenolic hydroxy compounds are easy to prepare, can be well separated and have other good chromatographic properties[190,350,511]. Their application is very important with polyhydroxy compounds of natural origin, such as hydroxy-[542] and polyhydroxyanthraquinones[197], gossypol[385], morphine[15,70,373,588], flavonoids[195,315], aminochromes[257] and others[516]. The use of trimethylsilylation with many other hydroxyl compounds has been quoted in Pierce's monograph[449].

3.1.5. Derivatives of optically active substances

Pereira *et al.*[442] employed *R*-(+)-1-phenylethyl isocyanate for the preparation of carbamates of optically active C_4–C_{10} alcohols. The individual derivatives of diastereoisomers have sufficiently different retention times to be resolved by GC. Other esters have also been used for this purpose. Anders and Cooper[14] described esters of 3β-acetoxy-Δ^5-etienic acid, and Hammarström and Hamberg[239] studied D-phenylpropionates of higher alcohols. Brooks *et al.*[73] employed sesquiterpenic drimanoyl chloride and monoterpenic chrysanthemoyl chloride for the preparation and resolution of esters of enantiomers of alcohols.

3.2. Aldehydes and ketones

In a similar manner to blocking amino groups by condensation with a carbonyl group, aldehydes and ketones can be modified for GC analysis by reaction with a suitable amino compound. The carbonyl group can also be oxidized when determining the resultant ester, or converted into an acetal or ketal.

3.2.1. Enamines

The preparation of some enamines of di-*n*-hexyl and di-*n*-heptyl ketones and their chromatographic properties have been described by VandenHeuvel *et al.*[562]. They prepared, in a medium of ethyl acetate and with catalysis by acetic acid, condensates with N-aminopiperidine, N-aminohomopiperidine, pentafluorophenylhydrazine and phenylhydrazine.

2,4-Dinitrophenylhydrazones (DNPHs) of carbonyl compounds have frequently been applied in isolations prior to GC analysis. Ralls[460,461] prepared the 2,4-DNPHs of mixtures of aldehydes and ketones and, after warming the condensates with α-ketoglutaric acid, the carbonyl compounds released were separated by GC. This method was developed further by other workers[150,236,377]. Jones and Monroe[300] employed dimethylaminobenzaldehyde to liberate the carbonyl compound from 2,4-DNPH. Halvarson[235] described an apparatus for the regeneration of carbonyl compounds and their determination on a microanalytical scale. Gadbois and coworkers[198,199] prepared 2,4-DNPH derivatives by reaction with Girard T reagent and released the carbonyl compounds by using an excess of formaldehyde produced from paraformaldehyde or methylolphthalimide. Employing the above method, Ronkainen and Brummer[470] analyzed α-hydroxyketones. Their 2,4-DNPHs were pre-purified by steam stripping in the presence of concentrated sulphuric acid. The diketones produced on reaction with oxoglutaric acid were injected into the gas chromatograph.

The direct GC analysis of the 2,4-DNPHs of aldehydes and ketones has also been described[177,310,520]. These derivatives display good chromatographic properties and permit a high sensitivity of analysis to be attained. Johnson and Hammond[297] used 2,4,6-trichlorophenylhydrazine as a reagent. Employing these derivatives with the ECD, it was possible to detect 10^{-7}–10^{-10} g of carbonyl compounds. The direct separation of 27 carbonyl compounds by programmed-temperature GC has been described by Jack and Riess[289].

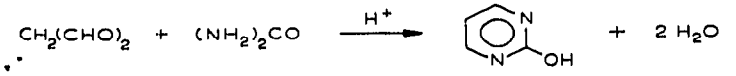
3.2.2. Oximes

Oximes are used almost entirely in special cases. Vogh[570] used them in the analysis of exhaust gases and utilized their acidic properties for their preliminary

isolation; hydrocarbons and other interfering components were extracted into pentane. However, oximes decompose to the corresponding nitriles[363] during GC, especially on contact with metals or their oxides. The separation must therefore be carried out in glass apparatus.

3.2.3. Other derivatives

Hamberg *et al.*[237] isolated and identified malonaldehyde after its condensation with urea, and converted the 2-hydroxypyrimidine produced, which has a low volatility but is stable, into a TMS ether. The reaction is



This method has been applied to the analysis of biological samples.

Schogt *et al.*[489] oxidized higher aliphatic aldehydes with silver oxide to the corresponding acids, which were subsequently separated after their esterification with diazomethane. Gray[221] synthesized dimethylacetals by refluxing aldehydes with a methanolic solution of hydrochloric acid and attained yields of over 95%. Acetals were oxidized to acids with chromium trioxide in glacial acetic acid and the acids were methylated with methanolic hydrochloric acid[222].

3.3. Amines

The GC of free amines, without special modifications to the column, is unsatisfactory owing to the spurious adsorption of the solute and the resulting peak tailing. Most commonly, the amino group is blocked by acylation, trimethylsilylation and the preparation of various condensation products (Schiff's bases).

3.3.1. Acyl derivatives

Anders and Mannering[15] described the chromatographic behaviour of acetyl and propionyl derivatives of substances that contain amino and hydroxy groups. They prepared the derivatives directly on the column by injecting the anhydride. In this way, it is possible to characterize some organic bases satisfactorily, but it is not possible to use this method for quantitative analysis. Nevertheless, Marmion *et al.*[372] described the application of this method to the determination of trace amounts of 2-naphthylamine in 1-naphthylamine by using a method of comparison with standards. Halogenated acyl derivatives have shown a much greater significance. A comparison of their properties and ECD responses was made by Clarke *et al.*[108]. It follows from their work that each species of derivative can be employed with certain species of amino compound; the highest sensitivity of analysis was achieved with HFB derivatives (Table 4).

Owing to their ease of preparation, trifluoroacetyl (TFA) derivatives of amines have great significance and have been used in the GC of aliphatic[124,411], alicyclic[360] and aromatic[104,151] amines and diamines[82,365,405]. This method has also been employed in the determination of biological amines in natural materials[50,109,316,422]. In a similar way, mono- and disubstituted ureas were determined[173]. The determination of compounds that contain several different functional groups is performed mostly through combination with other derivatives. Thus, Mori *et al.*[403] determined the

TABLE 4

COMPARISON OF THE CHROMATOGRAPHIC PROPERTIES OF SOME ACYL DERIVATIVES OF AMINES

Conditions, A: 6 ft. × 4 mm I.D. glass column; 6% QF-1 on Anakrom ABS, 60–70 mesh; column temperature 152°; carrier gas (nitrogen) flow-rate, 30 ml/min. Conditions, B: column as in A; temperature 155°; carrier gas flow-rate 80 ml/min.

Amine	*Derivative*	*Conditions*	*Retention time (min)*	*Peak shape*	*Sensitivity of determination**
Benzylamine	Acetyl	B	2.5	Asymm.	0.04
	Monochloroacetyl	B	3.0	Slightly asymm.	30
	Trifluoroacetyl	A	1.6	Symm.	0.8
	Pentafluoropropionyl	A	2.2	Symm.	229
	Heptafluorobutyryl	A	2.3	Symm.	715
α-Methylbenzylamine	Acetyl	B	2.5	Asymm.	0.16
	Monochloroacetyl	B	2.8	Slightly asymm.	32
	Trifluoroacetyl	A	2.2	Symm.	0.5
	Pentafluoropropionyl	A	2.2	Symm.	0.5
	Heptafluorobutyryl	A	2.2	Symm.	563

* Expressed as peak height (mm) per 10^{-9} mole of the compound.

products of the hydrolysis of copolyamides (diamines, diacids, amino acids) after their prior esterification with methanol–hydrochloric acid and acetylation with trifluoroacetic anhydride. Noguchi *et al.*[426] determined the metabolites of tryptophan in the form of their methoxy-TFA derivatives. Pentafluoropropionyl[17,314] and pentafluorobenzyl[401] derivatives were employed in the determination of trace amounts of amines in biological samples. Ephedrines[126], melathonines and indoleamines[138] and other biological amines[77] have been analyzed as their HFB derivatives.

3.3.2. Trimethylsilyl derivatives

The amine group is not very reactive in silylation reactions and is relatively difficult to silanize. In a mixture of hexuronic acid, 1-octanol and 1-octylamine, the amine gives the lowest yields[379] under different silylation conditions. However, the differences in reactivity are not significant and a number of amines can be chromatographed in the form of their silyl derivatives[449].

The above derivatives are prepared by the use of various silylation agents, mostly the more powerful agents. HMDS alone is usually inadequate for derivatizing secondary amine groups and is used in admixture with catalysts[181]. BSA is often employed[393], also with the addition of a catalyst. Butts[87] employed a mixture of BSTFA and 1% of TMCS for the preparation of the derivatives of amines and other compounds and tabulated their retention data. Maruyama and Takemori[375] prepared TMS derivatives of dopamines and related compounds by reaction with trimethylsilylimidazole in acetonitrile.

A more complicated situation is encountered with compounds that contain several amino groups of different types. Holmstedt *et al.*[270] studied the preparation of the TMS derivatives of tryptamine and related compounds. On applying BSA in

pyridine, mainly monosubstituted derivatives are produced, while the use of BSA–TMCS mixtures yields disubstituted derivatives as the major products. When a longer reaction time is allowed, however, comparable amounts of di- and trisubstituted derivatives are produced[559]. Albro and Fishbein[7] silylated metabolites of thyrosine and tryptophan with different agents. From the viewpoint of quantitativeness of the reaction and uniformity of the products, the most suitable agents are BSTFA and TMCS in pyridine, with the addition of TMSDEA if necessary.

In the molecules of catecholamines one or several hydroxy groups are present together with a primary or secondary amine group. Without blocking these polar groups, catecholamines cannot be analyzed by GC. Horning *et al.*[281] found that BSA and BSA–TMCS silylate the primary amine group to the second degree while the secondary amine group is converted slowly or not at all. In order to obtain uniform products, they employed TMSIM in acetonitrile, which silylates hydroxy groups. Upon subsequent addition of BSA–TMCS, primary amine groups are converted into bis-TMS derivatives while secondary amine groups remain unchanged. The amine group can also be blocked after silylation by TMSIM in another way. Horning *et al.*[279] used N-acylation with N-acetyl- and N-heptafluorobutyrylimidazole. Good results were obtained by blocking the amine group by condensation with a carbonyl compound[320]. Fig. 5 shows the chromatogram of a mixture of catecholamines. After trimethylsilylation of the hydroxy groups, the amine groups were converted into enamines by reaction with acetone. The secondary groups were not blocked[91].

3.3.3. Dinitrophenyl derivatives

These derivatives are used for the detection of trace amounts of amines with an ECD. Employing these derivatives, Weston and Wheals[582] determined about 1 ppm of cyclohexylamine in beverages and other materials. A more detailed study on the preparation and behaviour of these derivatives in GC has been described by Walle[573]. They are prepared by reaction with 1-fluoro-2,4-dinitrobenzene in benzene. Minimum amounts that can be determined lie in the range 2–20 pg. Good results were obtained by Edwards and Blau[163], who used 2,4-dinitrobenzenesulphonic acid for the preparation of these derivatives. Hydroxy groups present in various amines were blocked by silylation with BSA.

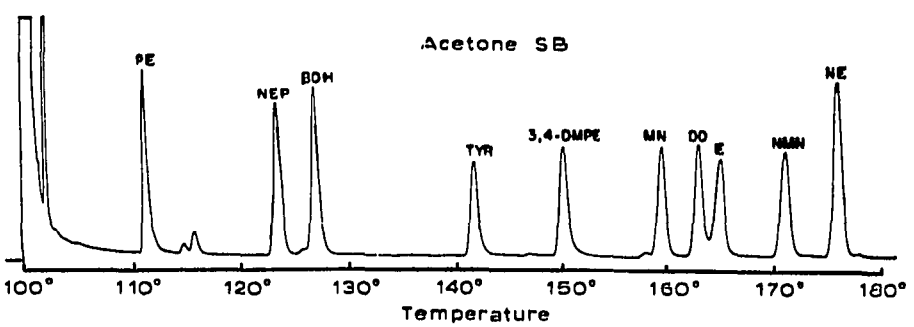


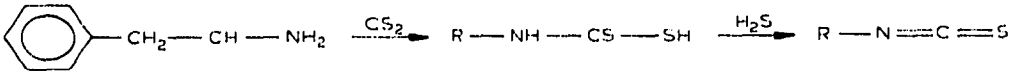
Fig. 5. Chromatogram of a mixture of catecholamines after their trimethylsilylation and conversion into enamines[91]. PE = β-Phenylethylamine; NEP = norephedrine; βOH = β-hydroxy-β-phenylethylamine; TYR = tyramine; 3,4-DMPE = β-(3,4-dimethoxyphenyl)ethylamine; MN = metanephrine; DO = dopamine; E = epinephrine; NMN = normetanephrine; NE = norepinephrine. Conditions: 10% F-60, temperature programming at 1.5°/min.

3.3.4. Condensation products

Umeh[554] separated and determined the isomers of aniline and toluidine, after their reaction with formic acid, in the form of formanilide and formtoluidide. Moffat and Horning[400] used condensation with pentafluorobenzaldehyde in order to prepare the derivative of phenethylamine, which shows the highest sensitivity in detection with the ECD; it was possible to determine as little as 10 pg of the amine.

3.3.5. Isothiocyanates

These compound are prepared by the reaction of the primary amine group with carbon disulphide and have good chromatographic properties. Brandenberger and Hellbach[65] resolved amphetamine and its methyl derivative by this means and applied the method to the determination of these substances in urine. The reaction proceeds as follows:



These derivatives were studied with various biogenic amines by Narasimhachari and Vouros[420,421], who published their chromatographic and mass-spectrometric data.

3.3.6. Other derivatives

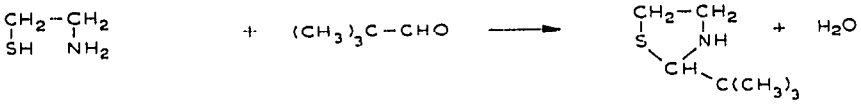
Gejvall and co-workers[213,471] separated and determined several low-molecular-weight amines after their conversion into urethanes by reaction with diethyl dicarbonate:

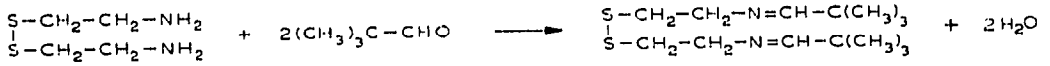
$$R\text{–}NH_2 + C_2H_5O\text{–}CO\text{–}O\text{–}CO\text{–}OC_2H_5 \rightarrow R\text{–}NH\text{–}CO\text{–}OC_2H_5 + CO_2 + C_2H_5OH$$

Dee[137] determined hydrazine and methylhydrazine as the corresponding pyrazoles after their reaction with acetylacetone. Neurath and Lüttich[424] converted asymmetric hydrazines into 5-nitro-2-hydroxybenzal derivatives. Sen[497] determined nitrosamines after their oxidation to nitramines with hydrogen peroxide. Frére and Verly[189] oxidized amines in aqueous solutions with an iodate and separated chromatographically the aldehydes produced. Hucker and Miller[283] chromatographed tertiary amines and compared their properties with those from the Hofmann reaction. These products are separated much better than the free amines and no tailing of the peaks occurs. Jenden and co-workers[244,295,296] analyzed quaternary choline and acetylcholine after their demethylation with benzene thiolate in butanol at 80°, injecting the tertiary amine into the gas chromatograph. The method was applied to the determination of choline and acetylcholine in biological materials. In order to separate optical isomers, Beckett and Testa[40] used N-TFA-L-prolyl derivatives prepared by the reaction of amphetamines with N-TFA-L-prolyl chloride. Corbin and Rogers[115] resolved the TFA, PFP and HFB derivatives of enantiomers of secondary amines on an optically active stationary phase, the ureide of L-valine isopropyl ester.

3.4. Sulphur compounds

Thiols require more drastic conditions for silylation. 1-Butanethiol does not react with an HMDS–TMCS mixture even under reflux, and the sodium or lead salts of saturated thiols have to be used in order to prepare their TMS thioethers[348]. Jellum *et al.*[294] analyzed cystine, cysteamine and other biologically important amines in the form of the thiazolidines and neopentylidines, after their reaction with pivaldehyde:





Barron and Mooney[36] chromatographed thioesters after their reduction with sodium borohydride. The alcohols produced were extracted and determined either directly or after their derivatization. Burchfield *et al.*[83] determined 4,4'-diaminophenyl sulphone and 4-acetamidophenyl-4'-aminophenyl sulphone after their conversion into iodo or bromo derivatives. N,N-Dialkyldithiocarbamates were chromatographed after their S-alkylation with diazomethane. Ethyl and propyl esters were prepared by reaction with iodoethane and 1-iodopropane, respectively[431].

3.5. Carboxylic acids

The direct analysis of free carboxylic acids by GC has been studied thoroughly and the results were good[529]. However, the presence of a carboxyl group in the molecule of the solute necessitates either a modification of the column packing or the use of a special technique in order to prevent the spurious adsorption of solute in the column[425]. The carboxyl group can be blocked simply and efficiently by esterification, and the chromatographic separation of the esters does not present serious difficulties. With substituted carboxylic acids, such as hydroxy and keto acids (amino acids are discussed separately), the situation is further complicated by the presence of the other functional groups and chemical derivatization of such compounds is essential. Chemical conversion of the acid into another compound is used only rarely, in special instances. For instance, 3-methoxy-4-hydroxymandelic acid can be chromatographed after its reduction and conversion into the vanilyl TFA ester[139]. A detailed study on the GC of carboxylic acids and their derivatives was described by Supina[529].

3.5.1. Esters

Owing to the ease of their preparation and their good chromatographic properties, these derivatives are widely used and have been studied in great detail. Methyl esters are the most commonly used esters. With carboxylic acids, methylation has been carried out with diazomethane[399,456,487,488], methanolic hydrochloric acid[282,390,526], methanolic boron trifluoride[9,20,394,395,410] and boron trichloride[67], and by the pyrolytic decomposition of tetramethylammonium salts[31,152,153,466]. In the esterification of keto acids (Krebs cycle), the reactions are complicated by the by-products

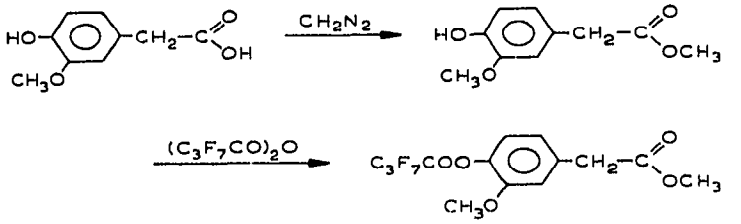
from the methylation of the enolized keto group[508]. When employing this method, Estes and Bachmann[172] observed decomposition of the derivatives in the column. Hautala and Weaver[256] employed boron trifluoride–methanol mixture for the esterification of the acids of Krebs' cycle acids, whereby pyruvic acid is converted into methyl 2,2-dimethoxypropionate. Felder *et al.*[179] used N,N'-dicyclohexylcarbodiimide in methanol for the preparation of the methyl esters of halobenzoic acids.

In general, it can be stated that methyl esters are suitable for the GC of higher fatty acids, *e.g.*, those produced by the hydrolysis of fats[298,359,361]. The methyl esters of lower fatty acids are volatile and may be subject to losses during their preparation. In order to perform the simultaneous determination of glycerol and fatty acids in the hydrolyzate of fats, Mason and co-workers[376,378] added 2,2-dimethoxypropane to the reaction mixture, thus converting glycerol into isopropylideneglycerol, which had good chromatographic properties. Some workers have employed higher alcohols for the esterification of lower acids. Dummel and Kun[155] described ethyl esters, Karmen[312] used 2-chloroethyl esters and Smith and Tsai[519] used trichloroethyl esters, which afford a selective analysis with the use of a halogen-sensitive detector. Appleby and Mayne[22] studied *n*-propyl esters and Thenot *et al.*[548] described their preparation by reaction with N,N-dimethylformamide dipropylacetal.

Butyl esters are prepared in a similar manner to methyl esters. Bezard and Bugant[49] employed butanol plus 2% of sulphuric acid and other workers[27,215,389] have used butanol saturated with hydrogen chloride and/or butanol plus boron trifluoride[341]. Wilcox[587] prepared the higher alkyl esters of hydroxybenzoic acids by reaction with diazoalkanes, catalyzed with 0.007% of boron trifluoride. In the absence of boron trifluoride, multiple peaks appeared in the chromatogram.

Benzyl esters[118,332] and *p*-substituted benzyl esters[578] have been used with short-chain fatty acids. Craig *et al.*[122] analyzed acids up to C_9 as their butyl and phenacetyl esters. Umeh[550] described the separation of the *p*-bromophenacyl and *p*-phenylphenacyl esters of C_2–C_{10} acids. In order to increase the sensitivity of analysis, Kawahara[319] used pentafluorobenzyl esters.

The methylation of phenolic hydroxy acids with diazomethane does not give uniform products owing to incomplete methylation of the hydroxy group[589]. Some workers[275,278,313] used trimethylsilylation in order to derivatize free phenolic and alcoholic groups. Sjöquist and Änggärd[512] chromatographed homovanillic acid after its conversion into the methyl ester of the heptafluorobutyrate:



Similarly, Dziedzic *et al.*[160] employed the hexafluoroisopropyl ester of the trifluoroacetate and obtained a high sensitivity with the use of an ECD. Anthranilic acid has been analyzed as the methyl ester of its trifluoroacetate[264].

3.5.2. Trimethylsilyl derivatives

With carboxylic acids, silylation agents react both with the carboxyl group

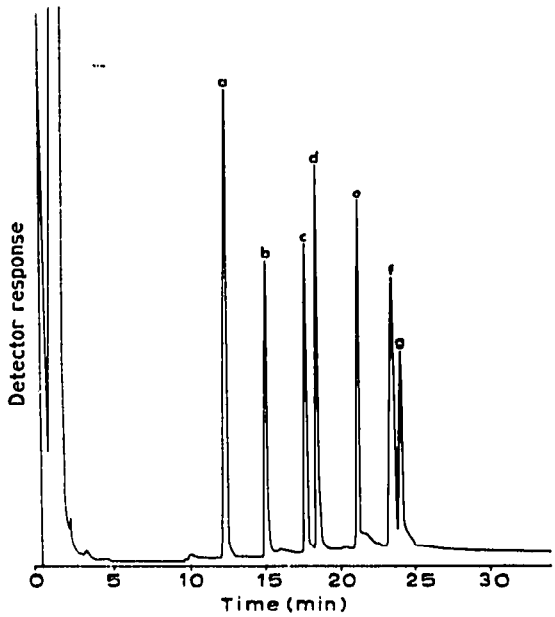


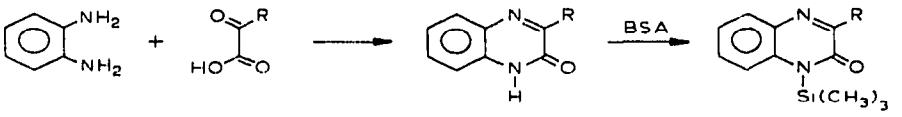
Fig. 6. Chromatogram of silylated phenolic acids[408]. a, *p*-Hydroxybenzoic acid; b, vanillic acid; c, syringic acid; d, coumaric acid; e, ferullic acid; f, sinapic acid; g, *n*-docosane. Conditions: 3% UCW-98 on Chromosorb W HP; temperature programming at 6°/min, initial temperature 100°.

proper and with other groups present in the molecule[449]. The use of these derivatives is especially advantageous in instances when acids are determined in the presence of other compounds that can also be silylated. Thus, Tallent and Kleiman[534] used BSA for the silylation of the hydrolyzate from lipids. The TMS esters showed good chromatographic properties and were separated from the ether of glycerol. Sato and Von Rudloff[484] determined saccharides and alcohols together with acids in the benzene extract from heart wood. In addition to the analysis of aliphatic acids[277,303,514,601], this method has been particularly useful with hydroxy acids[170,311,443,594] and polyhydroxy acids[446,504]. The complete silylation of all of the hydroxy groups present usually requires the catalytic action of TMCS. Aromatic acids can be converted by HMDS–TMCS into silyl derivatives only by refluxing the reaction mixture in toluene[317]. Better results were achieved by the use of BSA as a silylation agent[119]. The reaction is more rapid and the products are more uniform, even with phenolic acids[130,408] (Fig. 6).

If indolecarboxylic acids are to be silylated, it is necessary to use a stronger silylation agent in order to silylate the indolic nitrogen also. Good results are obtained with BSTFA in acetonitrile, and the separation of the TMS derivatives is better than that with the methyl esters[227].

In addition to the above TMS methyl ester derivatives, other combinations have also been used for the conversion of substitution derivatives of acids into volatile compounds. With Krebs' cycle acids, the use of TMS derivatives was only partially successful[472]. The ketonic group produces multiple peaks of keto acids (owing to enolization and decomposition in the column) and has to be blocked in a preliminarily stage[129]. The conversion of the ketonic group into an oxime with hydroxylamine and

subsequent silylation give derivatives that can be analyzed satisfactorily[271]. However, methoxime-TMS derivatives have been used more frequently[16,276,308]. Chalmers and Watts[96] have also applied ethyl and benzyl oximes successfully. Interesting derivatives were introduced by Hoffman and Killinger[267], which were later employed by other workers[192]. Keto acids are converted by reaction with aromatic *o*-diamines into the derivatives of chinoxalone, which, after silylation, can be chromatographed satisfactorily:



Scott[492] employed chlorosulphonic acid in order to block the hydroxy groups of hydroxy and hydroxyphenolic acids before their silylation with BSTFA. A better separation, compared with the TMS ester–ethers, was obtained with dihydroxy compounds in particular.

3.5.3. Dinitrophenylhydrazones

These derivatives can be used with acids that contain a carbonyl group, particularly with keto acids. Their importance is based mainly on the possibility of carrying out a preliminary separation of keto acids from the sample. Earlier, the chromatographic analysis was carried out after methylation and the liberation of the methyl esters from the 2,4-DNPH by ozonolysis[469]. However, the peaks of the by-products often overlap with the peaks of the keto acid esters and interfere with the analysis. Kallio and Linko[309] therefore chromatographed the 2,4-dinitrophenylhydrazones of methyl esters of keto acids directly. However, in this instance also, extra peaks due to isomeric derivatives occurred.

3.5.4. Anilides and toluidides

Umeh[555] used these derivatives successfully with lower carboxylic acids and described the conditions for their separation. The derivatives of formic acid can also be used for the separation and identification of the isomers of aniline and toluidine[554].

3.5.5. Cyclic boronates

n-Butyl boronates, which are usually used with difunctional compounds, can also be used with hydroxy and keto acids[75]. The advantage of these derivatives lies in the simultaneous separation of other difunctional compounds, such as diols, ketols and hydroxylamines.

3.5.6. Separation of optical isomers

As observed by Ackman *et al.*[1] and Annett[18], the enantiomers of hydroxy acids can be separated satisfactorily as their L-menthyl esters. Hammarström and Hamberg[239] used methyl ester D-phenyl propionates for the separation of the diastereoisomers of 3-, 15-, 16- and 17-hydroxyoctadecanoates. Poorer results were obtained with 2-, 14-, 4-, 7- and 13-hydroxyoctadecanoic acids.

*3.6. Amino acids**

The low volatility of amino acids, caused by the presence of a carboxylic and an amine group in the molecule, renders the GC analysis of free acids impossible. Compared with conventional analysis on ion exchangers, the use of chemical derivatives of amino acids has three substantial advantages: (1) the possibility of determining 10^{-4}–10^{-5} times smaller amounts of amino acids; (2) the possibility of shortening the time of analysis to as little as half an hour; and (3) the possibility of utilizing the instrumentation for other purposes. Nevertheless, the full utilization of these advantages is hindered by two general difficulties: (1) the quantitative preparation of suitable derivatives of all amino acids; and (2) the choice of a suitable selective sorbent on which all the derivatives will be well separated.

The difficulty in selecting a suitable derivative is due largely to the widely varying structures and chemical properties of amino acids. Difficulties are encountered particularly in the preparation of volatile derivatives of arginine, histidine and tryptophan. The acylation of the guanidine group of arginine and the imidazole group of histidine is complicated by the formation of salts in the strongly acidic medium that is necessary for the reaction. Also, the indolic nitrogen of tryptophan is difficult to acylate, and although the second centre of basicity is acylated easily, the monoacyl derivative produced is eluted from the column only at high temperatures. Lysine also needs a strongly acidic medium for the acylation of its second amine group. The derivatization of cysteine has to be carried out in an inert atmosphere, as cystine, which is produced on oxidation, forms a high-boiling derivative that is difficult to elute.

Other methods of blocking polar groups (*e.g.*, the preparation of TMS derivatives, dinitrophenylhydrazones and diisopropyl derivatives) are usually suitable only for a limited number of amino acids and/or in special instances. Detailed papers dealing with the problems of the preparation and properties of volatile derivatives of amino acids and with the application of the methods to biological samples have been published by Blau[53] and McBride and Klingman[382].

3.6.1. Acyl alkyl ester derivatives

These compounds are the most commonly used derivatives in the GC of amino acids. Different workers have used various combinations of the acyl and alkyl moieties; N-trifluoroacetyl-*n*-butyl esters proved to be most suitable. The possibility of using these derivatives in GC was demonstrated by Zomzely *et al.*[602] in 1962 and later by Lamkin and Gehrke[342]. Mussini and Marcucci[416] employed diazobutane for the esterification of amino acids; a more common method for the preparation of esters of higher alcohols is to carry out the reaction in the presence of anhydrous hydrogen chloride. With amino acids, this reaction is complicated by their low solubility in higher alcohols. Therefore, Blau and Darbre[56] dissolved the sample in a small amount of trifluoroacetic acid before the addition of the alcohols, and Stalling *et al.*[524] prepared butyl esters by transesterification of methyl esters, thus utilizing their solubility in butanol. Roach and Gehrke[148] successfully employed 3 *N* hydrochloric acid in *n*-butanol, shortening the time of reaction to 15 min.

* A more extensive treatment of this topic was given by Hušek and Macek, *J. Chromatogr.*, 113 (1975) 139.

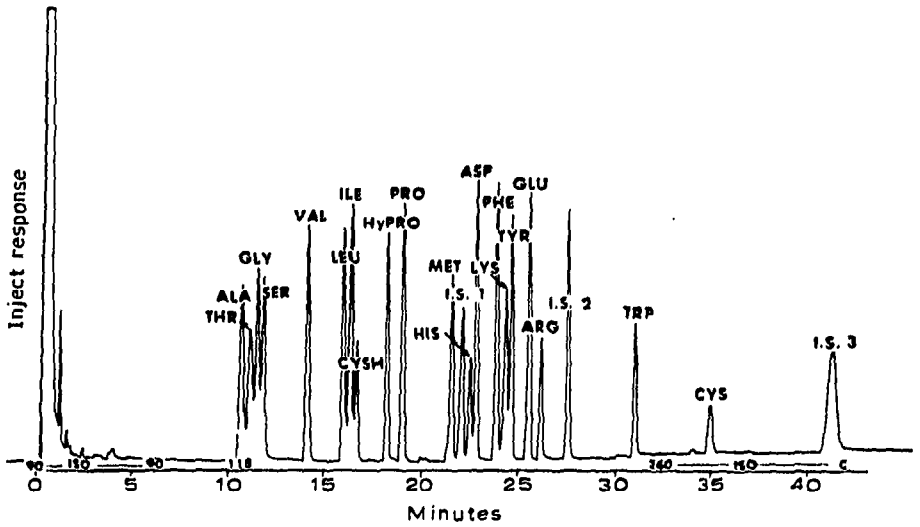


Fig. 7. Chromatogram of N-TFA-*n*-butyl esters of natural amino acids[210]. Conditions: glass column, 2.5 m × 2 mm I.D.; 10% Apiezon M on Chromosorb W HP, 80–100 mesh; temperature programming at 6°/min; initial temperature 90°. Internal standards (I.S.): 1, ornithine; 2, tranexamic acid; 3, *n*-butyl stearate.

The quantitative aspects of the preparation and determination of N-TFA-butyl esters of amino acids have been dealt with in several papers by Gehrke and co-workers[207,208,212] and McBride and Klingman[383]. As a result of their investigations, methods were developed for the macro, micro[201] and submicro[211] determinations of natural amino acids. The difficulties associated with the separation of the derivatives were obviated by employing two columns in series and mixed stationary phases[147,149,202,603]. More recently, Gehrke and Takeda[210] described the conditions for the separation of the derivatives of 20 amino acids on a single column (Fig. 7). Raulin *et al.*[464] described the application of this method to the analysis of a further 18 amino acids that are not present in proteins. Other workers have applied the method to the determination of amino acids in water[576], plasma[441] and other biological samples[92,260,339]. These derivatives can also be used for the resolution of optical antipodes[216,455,463], but with the *n*-butanol being replaced by *sec.*-butanol.

Compared with the above derivatives, N-trifluoroacetyl methyl esters of amino acids have the advantage of easier preparation, as amino acids are sufficiently soluble in the methanol–hydrochloric acid mixture, and no difficulties have been encountered in the derivatization[39,125]. However, Islam and Darbre[287] noticed that the methyl esters have a high volatility, which can cause losses during preparation, and developed an instrument[544] that enables the risk of such losses to be minimized. Although N-TFA-methyl esters have been used for the identification[54,370,584] and determination[111,231] of amino acids by some workers, these derivatives have not found wide application.

N-Trifluoroacetyl amyl esters were studied by Teuwissen *et al.*[545] and have been described in a number of papers by Darbre and Blau[55,56,131,132]. Compared with the methyl and butyl derivatives, amyl esters are less volatile and more stable, which

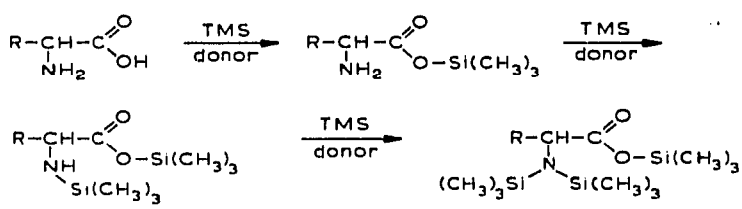
results in minimal losses during their preparation and analysis. However, the analysis is complicated by the incomplete separation of some of these derivatives, which restricts the application of the method; so far, this method has been used with only a limited number of amino acids[133].

Other combinations of the acyl and alkyl moieties have also been studied. The aims of the studies were to find more stable derivatives with shorter retention times that are easy to prepare and to enhance the sensitivity of analysis by employing highly fluorinated acyl anhydrides. Coulter and Hann[113] described the GC of N-acetyl-*n*-propyl esters of amino acids, and Pollock[454] studied pentafluoropropionyl and heptafluorobutyryl butyl esters, which have retention times that are as much as 35% lower than those of TFA-butyl esters and provide for selective detection with the ECD. Recently, remarkable results were obtained by Moss and Lambert[413] and Jönsson *et al.*[304] with N-HFB-*n*-propyl esters and by Zanetta and Vincendon[598] with HFB-isoamyl esters of amino acids. After esterifying the carboxyl group, Halpern *et al.*[234] protected the amine group by converting it into the N-thiocarbonyl group and achieved a good separation of the derivatives.

In addition to the above N-TFA-*sec.*-butyl esters, other derivatives have been employed for the separation of optical antipodes. In particular, N-TFA- and N-PFP-isopropyl esters[116,435–438] and N-TFA-2-octyl esters[216] have found application in this respect. Bonner[60] used N-TFA-S-prolyl methyl esters for the separation of *R*- and *S*-enantiomers of leucine. Fu and Mak[193,194] published a comprehensive comparison of different N-acyl alkyl derivatives of amino acids and the effect of the substituents on their chromatographic behaviour, and presented the retention data.

3.6.2. Trimethylsilyl derivatives

These derivatives were used in the GC of amino acids as early as 1960 by Birkofer and Ritter[51] and in 1961 by Rühlman[479] and Rühlman and Giesecke[480]. However, further work met with problems associated with the non-uniformity and instability of the products of silylation. The presence of different functional groups in the molecules of amino acids, displaying different reactivities toward silylation agents, results in the formation of different products, depending on the strength of the silylation agent employed and on the reaction conditions:



It was not until 1965 that Smith and co-workers[379,517] began to study the TMS derivatives of amino acids more systematically, investigating the efficiency of the individual silylating agents on model compounds. TMS-amines, particularly TMSDEA, were recommended as suitable reagents. Amides (BSA) give lower yields, and TMS-imidazole does not silylate amino acids[518]. Similar results were obtained by Klebe *et al.*[331], who introduced BSA as a strong silylation agent. The silyl derivatives that they prepared except for that of arginine, gave single sharp peaks, but decomposed

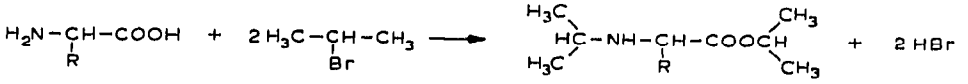
in the column. In addition, the mono-TMS-acetamide produced as a by-product during the reaction interfered with the derivatives of glycine and alanine. Mori *et al.*[404] applied BSA to the analysis of the products of the hydrolysis of copolyamides and determined the TMS derivatives of amino acids, diacids and diamines in their mixtures. In a similar manner, Caldwell and Tappel[90] separated sulpho- and selenoamino acids and the products of their oxidation.

Sharokhi and Gehrke[498] introduced the silylating agent bis-TMS-trifluoroacetamide (BSTFA) and Gehrke and co-workers[205,206] studied the conditions for the silylation of all natural amino acids. The higher volatility of the mono-TMS-trifluoroacetamide produced in the reaction permits the separation of this by-product from the derivatives of lower amino acids. However, the structure of the derivatives produced is strongly dependent on the reaction conditions[247]. For instance, glycine gives a single peak of the monosubstituted derivative when employing a solvent of low polarity (hexane, methylene chloride, chloroform, 1,2-dichloroethane) and two peaks of the di- and trisubstituted derivatives in more polar solvents[204]. Bergström *et al.*[46] studied the structure of the silyl derivatives of amino acids, prepared with BSTFA in the absence of a solvent. In this instance, glycine gave the mono-, di- and trisubstituted derivatives, lysine gave the tri- and tetrasubstituted derivatives and 6-aminocaproic acid gave the di- and trisubstituted derivatives.

In spite of the above difficulties, silyl derivatives have been employed in the determination of amino acids in biological samples[53,205,353]. Poclington[453] determined amino acids in the water of the Atlantic Ocean in this way. Dabrowiak and Cooke[127] separated the enantiomers of threonine as the TMS-N-TFA-D-(L)-prolyl methyl esters.

3.6.3. Diisopropyl derivatives

Pettitt and Stouffer[448] recently introduced promising derivatives. The reaction with 2-bromopropane leads to the isopropyl esters, but, at the same time, the isopropyl group is attached to the amine group:



The ε-amine group of lysine, the phenolic group of thyrosine and the thiol group of cysteine are also blocked. Only the hydroxy group of hydroxyproline is unreactive. Thus, the protection of almost all of the polar groups that occur in amino acids can be achieved by this single-step reaction. However, the different reactivities of different groups have an appreciable effect on the uniformity of the products and makes quantitative analysis difficult to perform[57]. Blessington and Fiagbe[58] used these derivatives for the identification of amino acids and amines in urine.

3.6.4. Dinitrophenyl derivatives

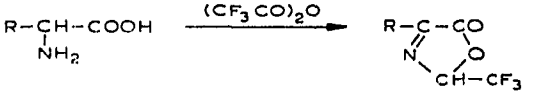
These derivatives can be used only for a limited number of amino acids, as the derivatives are not stable[452]. The carboxyl group is usually esterified with diazomethane[284] or by a mixture of methanol and thionyl chloride[398]. Although this method cannot be applied to all amino acids, it is useful for the determination of terminal amino acids in proteins[347,473].

3.6.5. Cyclic derivatives

The two functional groups in the molecule of an amino acid provide for the preparation of cyclic derivatives, the simplest of which are diketopiperazines[53]:

However, as they have two amide groups in the molecule, these compounds are not very volatile and also have other properties[535] that make them unsuitable for gas chromatography. Much better properties are displayed by phenylthiohydantoins, which were chromatographed successfully by Eriksson and Sjöquist[167] and Pisano *et al.*[452]. As these compounds are the final products of the Edman degradation of peptides, they provide for the rapid sequentional analysis of amino acids[344]. Nevertheless, these derivatives are not ideal as they require too high temperatures for elution (as high as 290°) and the derivatives of serine, threonine and cystine are unstable[53]. Some workers tried to overcome these difficulties by the preparation of methylthiohydantoins[26,174,451], N-TMS-phenylthiohydantoins[248,343,462] and N-TFA-phenylthiohydantoins[66,467].

The preparation of 2-trifluoromethyl-4-substituted oxazolones and their separation on a capillary column was described by Weygand[583]; recently, these derivatives were studied by Grahl-Nielsen and Solheim[220]. The derivatives are prepared by refluxing amino acids with TFA anhydride:



Some amino acids (serine and methionine, for instance) do not give these derivatives.

3.7. Thyroid hormones

As with amino acids, thyroid hormones also contain polar groups in the molecule, *i.e.*, carboxyl, hydroxy and amino groups, which have to be blocked prior to GC. Richards and Mason[465] esterified the carboxyl group by reaction with TFA anhydride and converted the ester into the N,O-bis-TFA methyl ester. Unfortunately, the authors did not give any quantitative data on the yield of the reaction and the sensitivity of analysis.

All of the polar groups in thyroid hormones can be efficiently blocked by single-step trimethylsilylation. Sharokhi and Gehrke[499] used both HMDS–TMCS and BSA in acetonitrile for this purpose. Good yields were obtained in both instances and a single peak for each substance appeared in the chromatogram. Other workers[8,29] obtained similar results. Jaakonmäki and co-workers[288,527] tried to obviate the instability of TMS derivatives towards humidity by preparing N,O-dipivalyl methyl esters, which are stable. They can be pre-purified by thin-layer chromatography[571] and give a sufficiently sensitive analysis with the use of an ECD.

3.8. Steroids

The choice and preparation of derivatives for the analysis of steroid compounds

are also complicated by the presence of different functional groups in the molecule. Four main polar groups, alcoholic, phenolic, ketonic and carboxylic, are encountered with steroids that occur in nature. Although some steroids are amenable to GC[428,429], chemical blocking of the polar groups is usually advantageous and sometimes essential. It improves the symmetry of the GC zones and the quantitativeness of their elution and usually increases markedly the volatility and stability of these compounds. The derivatization also permits some steric differences in steroids to be distinguished. An example is the separation of the epimers testosterone and epitestosterone, which can be well separated only after their conversion into TMS or acetyl derivatives[564]. The different retention characteristics of slightly differing steroids can be utilized for identification purposes[99,334]. Differences in the rate of derivative formation, caused by differences in steric hindrance[68], can be utilized in the same way. The selective formation of derivatives can be utilized advantageously for the removal of non-specific compounds from the sample[72,121]. If sub-nanogram amounts of steroids are to be analyzed, such as steroid hormones in the blood, for example, the preparation of derivatives that attract electrons is useful. Several workers have published comprehensive papers comparing the chromatographic behaviour of different derivatives of steroids and listing their retention data[164,229,406,564]. Baillie *et al.*[33] discussed the preparation and properties of the derivatives of corticosteroids and Knights[333] applied various derivatization methods to plant sterols. The separation of derivatives of steroids on a capillary column was demonstrated by Novotný and Zlatkis[429]. Berthou *et al.*[48] chromatographed derivatives of urinary steroids.

3.8.1. Silyl derivatives

The most commonly employed reaction for the preparation of volatile derivatives of steroids is silylation. Luukkainen *et al.*[367] were the first workers to utilize silyl derivatives of steroids in GC. A number of silylating agents have since been developed, TMCS, HMDS, TMSDEA and BSA being the most commonly used. When preparing the derivatives, it is necessary to observe the usual conditions of silylation reactions, which is difficult with biological samples and may lead to errors. A detailed discussion of these problems, together with valuable comments, has been presented by Pierce[449].

The currently most commonly used reagent, BSA, is so reactive that it converts all of the unhindered hydroxy groups (3-, 16-, *sec.*-17-, 20-, 21-) of steroids into TMS ethers without catalysis in several hours[272]. For the somewhat hindered 11β-hydroxy group, catalysis with TMCS is required in order to convert it into the TMS derivative[98]. The more powerful reagent BSTFA also reacts with the 11-hydroxy group, but the reaction proceeds slowly, so that the use of BSTFA without catalysis is not recommended. Strongly hindered groups (*e.g.*, 17α- in 17α,20α,21- or 17α,20β,21-triols) can be converted into TMS ethers only by using TMSIM in admixture with BSA–TMCS[98,481]. With digitoxigenin and related compounds, the reaction with TMSIM–BSA–TMCS mixture causes the lactone cycle to open and the enolized hydroxy group is also silylated[381], while HMDS silylates only the hydroxy groups of the skeleton[293,358]. Cowley *et al.*[120] used HMDS–TMCS–pyridine mixtures and studied the effect of the amount of solvent on the uniformity of the products. However, the use of this mixture causes complications due to the formation of a precipitate of ammonium salts[481].

In biological applications, it is rarely possible to use TMS derivatives

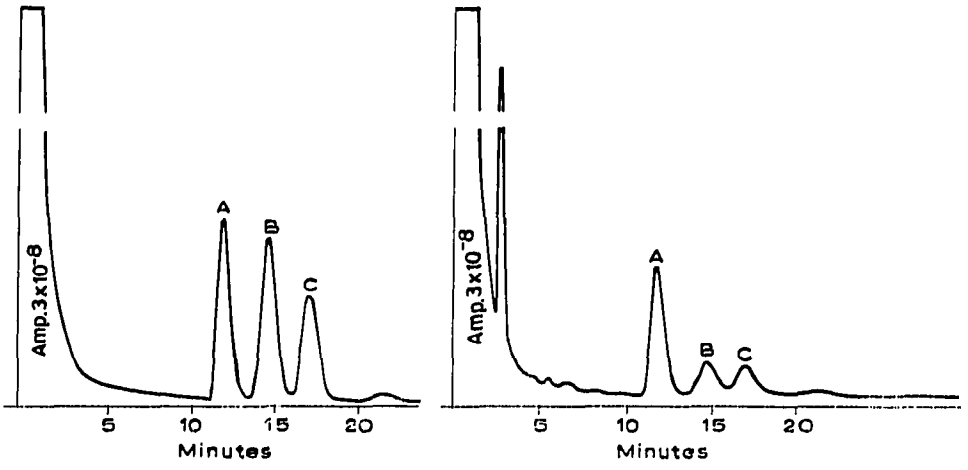


Fig. 8. Chromatograms of TMS derivatives of urinary 17-keto-steroids[47]. Left: model mixture of androstene (A), etiocholanolone (B) and dehydroepiandrosterone (C). Right: urine extract. Conditions: glass column, 1.8 m × 6 mm I.D.; 2% GE XE-60 on silanized Gas-Chrom P; temperature 225°, isothermal.

alone[76,101,230,510] (Fig. 8) and it is usually necessary to block other groups that are present. The use of oximes (see later) has proved useful[97,273]; Nambara and Bae[419] even employed a preliminary epoxidation of androstenes. The carboxyl group of bile acids has to be converted into its methyl ester by reaction with diazomethane prior to silylation[166]. Some suprarenal-gland hormones can also be determined in a similar way after their preliminary oxidation to 17α-carboxylic acids with periodic acid[330,392]. Fisher *et al.*[183] noted a marked instability of the TMS derivatives of vitamin D and related compounds. However, these difficulties do not occur with other steroids under anhydrous conditions. Chloro-, bromo- and iodomethyl dimethylsilyl ethers have also been described, but they have not found wide application[558,560]. They were studied systematically also by Eaborn and co-workers[162,219,550], who demonstrated their application to the determination of urinary steroids[551]. The derivatives yield a higher response with the use of the ECD[161,549] and characteristic mass spectra in combined GC–MS[100]. Morgan and Poole[402] employed trifluoropropyl, heptafluoropentyl and pentafluorophenyl dimethylsilyl derivatives for the selective determination of steroids from the group of ecdysones.

3.8.2. Acyl derivatives

Although the most important derivatives of this type are halogenated acyl derivatives, acetates of corticosteroids and sterols[71,427] have also been used successfully. Mougey *et al.*[414] resolved the epimers testosterone and epitestosterone as their acetates.

Landowne and Lipsky[346] and Brownie *et al.*[80] described the chromatographic behaviour of different haloacetates of steroids. However, because of their higher volatility and higher response in detection with the ECD, heptafluorobutyrates have found more general application[95,140,265,577,590]. Wotiz and co-workers[107,595] used these derivatives in the determination of steroids in biological samples. In order to deter-

mine trace amounts of steroids, Nakagawa *et al.*[418] prepared perfluorooctanoates and Kirschner and Taylor[328] prepared hexafluorononanoates and eicosafluoroundecanoates. The response of these derivatives is more than twice that of HFB derivatives. The preparation of acyl derivatives of steroids is easy, only Δ^4-3-ketosteroids giving a non-uniform product because of the enolization of the carbonyl group[140]. With polyfunctional steroids, acyl derivatives are used in combination with other derivatives, particularly with oximes[274].

3.8.3. Siliconides and acetonides

These derivatives come into consideration with steroids that contain two *vicinal* hydroxy groups. The acetonide derivative was utilized for the GC determination of estriol by Adlercreutz *et al.*[2]. Acetonides were also employed in analysis of corticosteroids[30]. Kelly[321,322] described the GC of the siliconides of corticosteroids that have hydroxy groups in the 16α,17α- or 17α,21-positions.

3.8.4. Bismethylenedioxy derivatives and cyclic boronates

As with siliconides, these derivatives can also be prepared from steroids with a dihydroxyacetonic side-chain. Bismethylenedioxy derivatives[327] and boronates[19] have been used with corticosteroids. Brooks and Harvey[74] described cyclic boronates with 17,20-diol, 20,21-diol and 17,20,21-triol steroids. The other free hydroxy groups on the skeleton were blocked by trimethylsilylation or acetylation.

3.8.5. Oximes

These derivatives are employed for protecting the carbonyl group of ketosteroids. Most commonly used are methoximes[176], which are usually combined with other derivatives of the hydroxy group, particularly silyl derivatives[261,280,546,547]. Benzyloximes[141,142] and pentyloximes and butyloximes[32] in combination with TMS derivatives have also been described. They have higher retention times and are therefore well separated from the related hydroxy compounds and possess good properties for use in combined GC–MS.

3.8.6. Hydrazones

These derivatives are prepared for the same purpose as oximes. Their preparation has been described by VandenHeuvel and co-workers[562,563]. Hydrazones can be used with advantage to study structural differences in ketosteroids by means of retention data. Pentafluorophenylhydrazones have an appreciable affinity for electrons and provide for the sensitive detection of steroids with a ketonic group in plasma[25].

3.8.7. Methyl ethers

The application of the methyl ethers of phenolic steroids with the hydroxy group on the third carbon atom was described by Brown[79]. Clayton[110] extended the use of this method to a further 30 steroids (estrogens). A more common application of this method is its combination with acetylation[121,391] or trimethylsilylation[3,366] for the routine determination of estrogens by GC.

3.8.8. Ketals

Sarfaty and Fales[482] prepared these derivatives by the reaction of the ketone

(usually a halogenated acetone) with the steroidic alcohol and methylating with diazomethane the hemiketal produced. The derivatives are sufficiently stable and are not hydrolyzed, even in aqueous solutions, but the sensitivity of their detection with the ECD is lower than that with HFB esters.

3.9. Saccharides and related compounds

The molecules of carbohydrates contain, in addition to hydroxy groups, other polar groups, such as carbonyl, carboxyl and amine groups. Their volatility is very low, so that they cannot be chromatographed as such. The polyfunctionality of carbohydrates makes the quantitative preparation of a uniform product difficult, and often as many as four peaks result from a single compound, which complicates the identification of the individual compounds. Hence, with mixtures the chromatograms are very involved and their interpretation is difficult. Nonetheless, silyl derivatives and methyl ethers of saccharides and related compounds are used in GC. A detailed treatment of these problems and a number of valuable references can be found in the papers by Sloneker[515] and Wells *et al.*[581].

3.9.1. Methyl ethers

The separation of mono-, tri- and tetramethyl ethers of sugars was described as early as 1958 by McInnes *et al.*[388]. Their results were promising and pentoses were easily separated from each other and from hexoses. However, the separation of hexoses and their anomers was unsatisfactory. Whyte[526] described the GC of methylated sugars and hexuronic acids. Kircher[325] employed a specially adapted column and separated successfully both the α- and β-anomers of methyl pyranosides from methyl furanosides.

The problem of the choice of the stationary phase, the degree of methylation and the application of the method to different carbohydrates have been dealt with by several workers[12,187,357]. Anderle *et al.*[13] described the separation of the optical isomers of xylofuranosides after their conversion into methyl ethers. As expected, derivatives that have a smaller number of methoxy groups have longer retention times, so that some monomethyl ethers have such long retention times that they are completely unsuitable for GC[581]. Therefore, when applying this method to disaccharides and polysaccharides, the products of their methanolysis have to be further modified. Kircher[325] methylated the polysaccharides with methyl iodide, while Bishop and Cooper[52] reduced the monomethyl ethers of sugars to the corresponding polyalcohols and analyzed them as their pentaacetates. A combination of methyl ethers with other derivatives, such as acetates and nitriles, has been used with sugars[61,345] and related polyalcohols[105].

3.9.2. Silyl derivatives

The preparation of the TMS derivatives of carbohydrates, which are polyhydroxy compounds, does not present any serious difficulties and they are completely silylated under mild conditions. Sweeley *et al.*[532] developed a simple versatile method, in which the silylation of a model compound, methyl α-glucopyranoside, was accomplished with HMDS–TMCS (2:1) in pyridine, which silylates all of the hydroxy groups of the substrate under normal conditions. Unless the reaction proceeds to completion, multiple peaks occur. They achieved an excellent separation even of the individual

anomers and configurational isomers of pentoses, hexoses, disaccharides and others. A modification of this method has also been used successfully by other workers for the preparation and GC of the TMS derivatives of glucose[114], glycosides[196,259,324], hexoses[543], heptoses[430] and other sugars[291,307,352] and related polyalcohols[159,188,225] and their oxidation products[143]. With some stationary phases, the pyridine produces a large tailing peak that can overlap some of the peaks of the derivatives, thus interfering with the determination of the latter. Lehrfeld[355] described a procedure for the removal of the pyridine from the mixture after the reaction has been completed and its replacement with *n*-hexane. Weiss and Tambawala[580] further simplified the method and carried out the reaction directly in aqueous solution. In this instance, the success of the determination consists in adding such a large excess of the silylation agent that all of the water is effectively removed.

The use of other reagents suffers from difficulties due to the formation of anomers. BSA in pyridine causes the anomerization of hexoses, and the chromatogram of a single sugar can contain four or five peaks[449]. In order to obtain uniform products, some workers oxidized simple sugars to the corresponding acids and their 1,4-lactones were converted into TMS derivatives, which were subsequently analyzed[409,445]. TMS derivatives have also been used successfully in the analysis of amino sugars by GC[457]. However, when employing a weaker silylating agent, only the hydroxy groups are silylated and the amine group remains unchanged[533]. Therefore, Perry[444] blocked the amine groups of some amino sugars by preliminary acetylation with acetic anhydride. The complete silylation of the phosphates of sugars is usually carried out with stronger silylation agents (BSA + TMCS and others)[249,500].

Halomethyldimethylsilyl and dimethylsilyl derivatives of carbohydrates are prepared under conditions the same as those for TMS ethers. In GC analysis, the halo compounds have retention times that are several times longer and their application is limited[531]. Dimethylsilyl ethers have retention times that are half of those of TMS derivatives and can be employed with advantage mainly with oligosaccharides[10].

3.9.3. Acyl derivatives

Acyl derivatives are used mainly with polyalcohols and sugars after their preliminary reduction. The method was introduced by Gunner *et al.*[228] and further developed by Sawardeker *et al.*[486]. Reduction with sodium borohydride and acetylation with acetic anhydride proceed quantitatively. Potential difficulties stem from the borate produced, which interferes in the acetylation, and from the pyridine solvent, which produces tailing peaks. Albersheim *et al.*[4] employed acetyl derivatives for the determination of sugars in cellular walls. Lehnhardt and Winzler[354] described the determination of sugars in glycoproteins (Fig. 9). Employing this method, Griggs *et al.*[226] determined neutral and amino sugars present together in mucins.

Excellent chromatographic properties are displayed by the acetates of aldonitriles of sugars, which are prepared by the reaction of sugars with hydroxylammonium chloride. The oxime produced is acetylated without its isolation and dehydrated to the nitrile by warming it in pyridine and acetic anhydride. Varma *et al.*[565] noted the long-term stability of these derivatives and applied the method to the analysis of natural material. They also analyzed in this way hexosamines after their deamination[232].

Vilkas *et al.*[569] described the successful separation of the TFA esters of mono-, di- and trisaccharides. The derivatives were prepared directly by the reaction of TFA

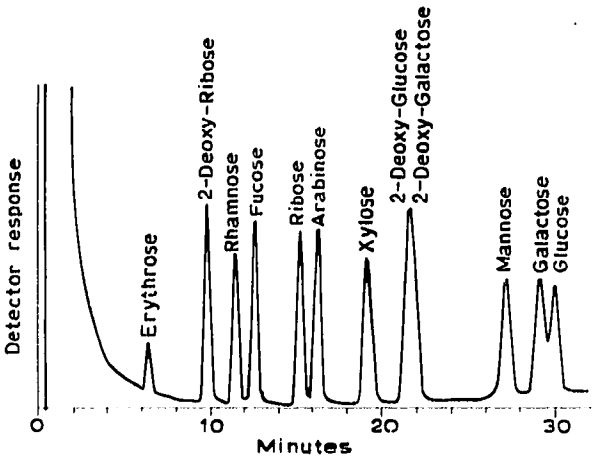


Fig. 9. Chromatogram of a mixture of sugars after their reduction and acetylation[354]. Conditions: glass column, 1.83 m × 4 mm I.D.; 0.75% Hi-EFF-1 BP, 0.25% EGSS-X and 0.1% 144-B (phenyldiethanolamine) on Gas-Chrom Q, 60–80 mesh; temperature programming at 1.3°/min, initial temperature 160°.

anhydride in acetonitrile with the carbohydrate. These derivatives are sufficiently thermostable and more volatile than TMS derivatives. In the acylation of amino sugars, the amine group is also blocked, which usually does not occur in silylation[597]. The application of these derivatives to sugars after their reduction to the corresponding alcohols was studied by Imanari *et al.*[286]. Anderle and Kováč[11] determined in this way mono-O-methyl-D-glucose after its reduction to monomethylglucitol and subsequent trifluoroacetylation.

3.9.4. Acetals, ketals and other derivatives

Kircher[325] described the successful GC of 4,6-O-ethylidene-D-glucose-1,2,3-O-triacetate. Adequate volatility for GC is also displayed by 1,2- and 5,6-O-isopropylidene derivatives. The chromatographic separation of these derivatives on a preparative scale was studied with 5- and 6-deoxyglucopyranose by Hedgley *et al.*[258]. A detailed study of the separation of acetal and ketal derivatives with free hydroxy groups or with the latter groups substituted with acetyl, benzyl, benzoyl, methyl or toluene-*p*-sulphonyl groups was published by Jones *et al.*[299].

3.10. Bases of nucleic acids, nucleosides and nucleotides

The direct GC analysis of the components of nucleic acids is rendered impossible by the presence of hydroxy and amine groups bound to the pyridine or purine core. Miles and Fales[396] used a combination of acetyl, methyl and isopropylidene derivatives in order to block the polar groups. They achieved a good separation of the products, but the peaks were asymmetric and showed considerable tailing. MacGee[368] studied the methyl derivatives and employed them for the determination of the ratios of the bases in nucleic acids. They were prepared by the thermal decomposition of tetramethylammonium salts in the injection port. The quantitative analysis

is hindered by the multiplicity of the products (there are as many as four peaks for adenine) and by the fact that different compounds may give the same derivative. For instance, the total methylation of xanthine, theobromine and theophylline results in caffeine in each instance.

A much wider use has been found for trimethylsilyl derivatives in the GC of nucleosides. Hancock and Coleman[243] prepared these derivatives by reaction with HMDS and TMCS in pyridine and obtained multiple products and asymmetric peaks. Later, Hancock[240] described the successful analysis of adenosine and showed that the TMS derivatives can be used for quantitative work. In a series of papers, Hashizume and Sasaki[251,254,483] described the GC separation of the TMS derivatives of some ribonucleotides and purine and pyrimidine bases. Employing phenanthrene as an internal standard, they obtained quantitative data for five main bases and demonstrated the application of the method to the determination of the ratio of bases in RNA and DNA.

Gehrke and co-workers[203,209] used BSA in acetonitrile as the silylating agent. They found optimum conditions for the preparation of uniform products; only cytosine and 3-methylcytosine gave two TMS derivatives. The five main bases were determined at the microgram level with a relative error of 3%. Further papers dealing with the GC of the derivatives of nucleosides are those of Hancock[241], Jacobson *et al.*[290] and Butts[85]. In the silylation of the derivatives of adenosine[241], small amounts of tetra-TMS derivatives were produced in addition to the stable tri-TMS derivatives produced by the silylation of the saccharide component. Jacobson *et al.*[290] successfully prepared TMS derivatives of guanosine and cytidine, which are among the nucleosides most difficult to silylate. Butts[85] converted the amine groups of nucleosides into methoximes by reaction with methoxylamine hydrochloride prior to silylation. The derivatives had shorter retention times and gave more symmetrical peaks than the TMS derivatives alone. The silylation of some less common nucleosides, *viz.*, those of dihydrouridine, pseudouridine, methylinosine, 6-thioguanosine and methylguanosine, was achieved with the use of BSTFA[242].

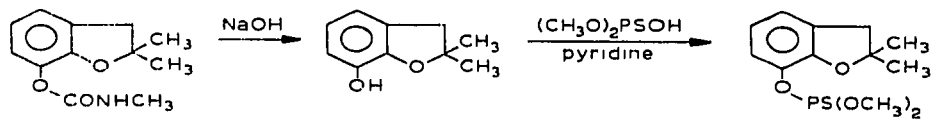
The application of TMS derivatives to the determination of the components of nucleic acids in their hydrolyzates has been described by different workers[203,254,340]. The hydrolysis is carried out with perchloric or formic acid and, after purifying the free bases on an ion exchanger, they are silylated and analyzed by GC. The method has been developed for work on the macro, semimicro and micro scales.

3.11. Insecticides and other pesticides

These substances comprise different types of chlorinated hydrocarbons, organophosphorus and -sulphur compounds, carbamates, heterocyclic derivatives and other substances. GC has proved very useful in the analysis of these compounds, and most pesticides have been analyzed successfully even in their free state[59]. The decomposition of chlorinated insecticides in the column and the presence of polar groups, especially hydroxy groups, in the molecules of some insecticides favours the preparation of chemical derivatives.

Chau and Cochrane[102,103] partially dechlorinated insecticides of the chlordane type with bases and converted them into uniform acetyl, TMS and/or *tert.*-butyl derivatives. This procedure gave single peaks with good shapes in the chromatogram

of each individual compound. Pionke *et al.*[450] analyzed directly insecticides that had been partially dechlorinated with potassium hydroxide, whereas endrin and dieldrin were saturated with hydrogen chloride prior to GC. The individual compounds were identified by chromatographing them after derivatization and in the free state on two stationary phases and comparing the retention data. Some workers blocked the phenolic and acidic groups of pesticides by acetylation[568], methylation with diazomethane[180] or trimethylsilylation[28,184]. In all instances the chromatographic properties were improved to such an extent that the chromatograms were amenable to quantitation. Diverse derivatives have been employed with carbamate insecticides. Bowman and Beroza[63] hydrolyzed carbamates with alkali-metal hydroxide to the corresponding phenols, which were further converted by reaction with dimethoxythiophosphate into the dimethoxythiophosphoryl derivative:



These derivatives display good properties for GC. Holden *et al.*[268] condensed the methylamine or dimethylamine group, liberated by hydrolysis, with 1-fluoro-2,4-dinitrobenzene and determined the resulting dinitroaniline with high precision using an ECD. Tatton and Wagstaffe[538] analyzed fungicides based on organomercury compounds. By reaction with an ethereal solution of diphenylthiocarbazone, the mercury present was converted into the dithizonates, which can be easily determined by GC.

3.12. Pharmaceuticals and drugs

The most suitable derivatives for use in the GC of these widely varying substances depend on the type of polar group present. Antibiotics are determined mostly as their TMS derivatives, which are useful because several different functional groups are often present in the molecule. Chloramphenicol and related antibiotics have been chromatographed by a number of workers[21,200,292,371]. As BSA does not give uniform products, HMDS–TMCS in pyridine is more suitable for the preparation of these derivatives. By employing a flame-ionization detector, concentrations as low as 0.2 μg/ml of these substances have been determined in biological materials. Tsuji and Robertson[552,553] have described a similar method for the determination of neomycins and attained high precision, despite the difficulty of hydrolyzing the derivatives. A number of penicillins have been analyzed in the same manner[266].

Vitamins comprise diverse chemical compounds and the range of the possible derivatives is also large[323]. Vitamin A has been chromatographed as its methyl and acetyl derivatives[156], but the instability of these derivatives necessitates the use of a special modification of the chromatographic column. The B vitamins are converted into acetyl, TFA or TMS derivatives prior to analysis[262,336,337]. With other vitamins, the polar groups in the molecule must also be blocked. Vitamin C was analyzed successfully as its TMS derivative[566] and vitamin D can be analyzed in a similar manner[575]. For the GC determination of vitamin E, conversion into the acetate was recommended[323].

Barbiturates can often be chromatographed, but they have long retention times and undergo strong adsorption in the column. These disadvantages do not occur with the methyl derivatives, which also give better resolution of the individual barbiturates. They are prepared either by reaction with a 10% solution of methanol saturated with potassium carbonate[374] or by pyrolysis of the tetramethylammonium[154,525] or trimethylanilinium[432] salts. Kowblansky *et al.*[338] resolved individual barbiturates and purines after their conversion with tetra-*n*-butylammonium hydroxide into the butyl derivatives. Parker *et al.*[434] identified barbiturates and other substances by utilizing the characteristic shifts in the retention times of the derivatives in comparison with those of the parent compounds.

Of the many other pharmaceuticals, we may mention the GC determination of narcotics as the acetyl and propionyl derivatives[415] and, of special interest, the determination of the analgesic pentazocine after its conversion into the pentafluorobutyryl ether[78] and of cambendazole after its silylation with BSA[561]. A more detailed survey of the GC analysis of pharmaceuticals and their derivatives has been presented by Kern *et al.*[323].

3.13. Anions of inorganic acids and related compounds

Anions of inorganic acids can be converted into volatile TMS derivatives and determined by GC. Following the work of Hashizume and Sasaki[252,253], who prepared the TMS derivatives of phosphates and glycerophosphates in very good yields by reaction with HMDS–TMCS, Butts[86] and Butts and Rainey[89] extended the method to other inorganic anions. Sodium and potassium salts are only slightly soluble in the derivatizing agent, thus giving low yields of TMS derivatives, and ammonium salts are more suitable. The above workers described the preparation and separation of the TMS derivatives of borates, carbonates, phosphates, arsenates, vanadates and other anions. Zinbo and Sherman[600] analyzed the TMS derivatives of phosphates by combined GC–MS. Wu *et al.*[596] identified the TMS esters of silicates by this means. Mattheuis *et al.*[380] determined trace amounts of phosphates in water after extraction of the ammonium salts with octanol–toluene and their subsequent trimethylsilylation. Boyden and Clift[64] used TMS derivatives in order to resolve phosphates and different mono-, di- and trialkyl-substituted phosphates. The TMS esters of aminoalkyl phosphates are not very stable and, therefore, the amine group must be blocked by converting it into an N-acetyl, isocyanate or similar derivatives[250].

Substituted anions of mineral acids can be determined by GC after their conversion into methyl esters. Taulli[539] resolved the isomers of hexadecene-1-sulphonic acid by the GC of their methyl esters, which were prepared with diazomethane. Various alkyl phosphates[128,246] have been determined in a similar manner.

Sulphonic acids are determined by GC as their methyl esters[45] or chlorides[263,326,440]. Nagai *et al.*[417] analyzed alkenyl and hydroxyalkyl sulphonates after their hydrogenation and conversion into sulphonyl chlorides. Parsons[439] showed that sulphonyl chlorides decompose in the column and are therefore unsuitable for GC separation. He converted sulphonyl chlorides into fluorides which are substantially more volatile and thermostable, by reaction with potassium fluoride. Shimoishi and Tôei[502] determined trace amounts of selenium in sulphuric acid by its oxidation to selenite and further conversion with 4-nitro-*o*-phenylenediamine into 5-nitropiaselenol.

The toluene extract of this derivative was chromatographed with high sensitivity with the use of an ECD. Srinivasan *et al.*[522] employed butylamine for the isolation of sulphates. After its isolation, the derivative is treated with an alkali-metal hydroxide and the butylamine liberated is determined by GC. MacGee and Allen[369] determined halide anions by converting their alkali-metal salts to tetraalkylammonium salts, which decompose to trialkylamines and alkyl halogenides upon injection into the gas chromatograph. In the determination of fluorides, the alkyl fluoride gives hydrogen fluoride and the corresponding olefin.

3.14. Miscellaneous

Durbin and Zlatkis[158] determined pyridine and quinoline bases after their reduction with hydrogen and conversion of the unsaturated compounds into pentafluoropropionates. The peaks were sufficiently symmetrical, and the sensitivity of analysis was increased by using an ECD. Ingle *et al.*[285] used the TMS ethers of the hydroxy derivatives of the above bases. TMS derivatives were also employed in the GC separation of pterins[362], coumarin and hydroxycoumarin[134]. Because of their higher volatility, TFA esters were also recommended for the GC of coumarin and its derivatives[135].

Slatter[513] determined diketopiperazines after their reaction with TFA anhydride. He resolved successfully all of the *cis*- and *trans*-isomers and diastereoisomers. Estas and Dumont[171] employed thermal degradation of tetramethylammonium salts for the methylation of hydantoins and their GC determination in biological samples. In comparison with other methods, this procedure has the advantages of specificity, sensitivity and speed of analysis. Glowacki *et al.*[218] determined 1,2-epoxides after their reaction with methanolic boron trifluoride or reduction with aluminium lithium hydride. In both instances, two products were obtained, but the ratio of their amounts was constant and the chromatograms could be evaluated quantitatively. Fioriti *et al.*[182] treated epoxyglycerides by reaction with ketones in the presence of boron trifluoride and chromatographed the derivatives of 1,3-dioxolane produced. Cyclopentanone was shown to be the most suitable starting material for the preparation of these derivatives. McDonough and George[386] separated *cis*- and *trans*-isomers of olefins after their stereospecific epoxidation with *m*-chloroperbenzoic acid. The oxirane derivatives of the *cis*- and *trans*-isomers have different retention times and can be well separated. Hasty[255] described the GC determination of iodine by its reaction with acetone–sulphuric acid (1:1 molar proportions), extraction of the reaction mixture with hexane and injection of the extract into the gas chromatograph. The iodoacetate obtained is sufficiently stable and can be well separated and detected.

4. ABBREVIATIONS USED

BSA	N,O-Bistrimethylsilylacetamide
BSTFA	N,O-Bistrimethylsilyltrifluoroacetamide
DMS	Dimethylsilyl (derivative)
DNPH	Dinitrophenylhydrazone
ECD	Electron-capture detector
FID	Flame-ionization detector

GC	Gas chromatography
HFB	Heptafluorobutyrate
HMDS	Hexamethyldisilazane
HMDSO	Hexamethyldisiloxane
PFP	Pentafluoropropionate
TFA	Trifluoroacetate
TMCS	Trimethylchlorosilane
TMS	Trimethylsilyl (derivative)
TMSDEA	Trimethylsilyldiethylamine
TMSIM	Trimethylsilylimidazole

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